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EFFECT OF PROLONGED AND INTERMITTENT SULFONAMIDE FEEDING ON THE BASAL METABOLIC RATE, THYROID AND PITUITARY[1]

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Submitted for publication December 22, 1943

In two preliminary notes, we reported marked thyroid enlargement and hyperplasia in rats and mice as the result of feeding sulfonamides, and the highly active compound, thiourea (1, 2).

The degree of thyroid enlargement has been shown by us (3) to be directly proportional to the blood level of the compound employed, inversely proportional to the age of the animal, and a logarithmic function of the time. The degree of colloid loss and hyperplasia cannot be inferred from the size of the gland alone. Generally speaking, the histologic changes are produced by a lower level of drug than is the increase in size. The appearance of thyroidectomy cells in the pituitary closely follows these thyroid alterations.

The all-important problem of the nature of the basal oxygen consumption was answered by demonstrating in a carefully controlled experiment (3) that 2% sulfaguanidine produces a fall in the B.M.R. of 10% in 5 to 7 days, when approximately one-half of the colloid has disappeared; and, of 20% in 10 to 14 days, when all but traces of colloid have vanished. The thyroid-metabolic effect of these compounds is not prevented by iodine (in some cases it is increased), thus distinguishing them from hitherto known goitrogenic agents. However, all of the effects are prevented by the daily administration of as little as 1 gamma of thyroxin per 10 gm. of body weight. The response in the B.M.R. of the highly sensitive thyroidectomized rat to 0.7 microgram of thyroxin per 10 grams of body weight is not inhibited by 2% of sulfaguanidine, nor does this compound further lower the B.M.R. of thyroidectomized rats. These observations favor the hypothesis that the drugs neither destroy thyroxin, nor increase the

[1] Supported by grants from the Rockefeller Foundation and the Williams-Waterman Fund of the Research Corporation.

tissue requirements for the hormone, but rather inhibit its formation. Thyroidectomy cells are found in the anterior pituitary, in the absence of gonadal degeneration, and, hence, presumably as a result of thyroid hypofunction. It is postulated accordingly that the anterior pituitary in its turn produces histological changes in the thyroid. As was to be expected, hypophysectomy prevents the thyroid enlargement (3).

The thyroid enlargement found in rats chronically poisoned with phenyl thiourea was ascribed by Richter and Clisby (4) to a fall in body temperature. Astwood et al. (5) observed a decrease in growth and food consumption in rats fed sulfaguanidine, but were unable to demonstrate a fall in the B.M.R. within 2 weeks. However, prolonged treatment or exposure to cold did result in a depression of the B.M.R. by sulfaguanidine or thiourea. The incorporation of 0.1% thyroid powder in the diet prevented thyroid enlargement. Non-toxic levels of iodine were ineffectual.

The increase in the B.M.R. and the toxic effects produced by the high level of 1% thyroid powder were not prevented in intact rats by 2% sulfaguanidine. In hypophysectomized rats, the loss of weight and ultimate death effected by 0.1% thyroid powder was not prevented by sulfaguanidine. The prevention of these toxic reactions led them to also infer that the drugs neither destroyed nor antagonized the action of the thyroid hormone, but prevented its formation. The failure of the drugs to produce thyroid enlargement in hypophysectomized rats was taken as evidence that this gland causes the thyroid enlargement in the intact drug fed animal.

Three cases of hyperthyroidism have been successfully treated by Astwood with thiourea and thiouracil, a compound found by him to be particularly potent in enlarging the thyroid gland of rats (6, 7). The B.M.R. began to fall in 7 to 14 days. Soon after, another group of Boston workers, Williams et al. (8) also reported favorably on the treatment with thiouracil of 9 cases of hyperthyroidism with an average B.M.R. of +63. The B.M.R.'s reached normal levels in 3 to 7 weeks.

In view of this recent clinical application of these compounds and because of the widespread use of the sulfonamides in the armed forces, we wish to report the effects on the thyroid and pituitary glands, and on the B.M.R. of protracted and repeated administration of sulfaguanidine. Particular interest also attaches to the status of the B.M.R. following the cessation of prolonged drug administration.

METHODS

In an earlier communication (3), methods employed to test the goitrogenic activity of the sulfonamides and thioureas have been described in detail. Although weanling rats, 21 to 23 days of age, and weighing 35 to 50 grams, were used most extensively in the present experiments, a large number of growing and old adult animals were also employed. Sulfaguanidine was incorporated in the McCollum stock diet with the aid of a mechanical rotary mixer, thus assuring its uniform distribution. The animals were given a solution of iodine water once weekly containing 20 mg. iodine and 40 mg. potassium iodide per liter. They were housed in individual animal cages and allowed to consume the diet ad libitum. The animals were killed with chloroform and the thyroids dissected and immediately weighed on a torsion balance. The time elapsing between the removal of the thyroid and its weighing was less than 30 seconds. Zenker-formol was used as the fixative for all tissues. The paraffin sections were stained with hematoxylin and eosin. The pituitary was stained with an adapted Mallory connective tissue stain which sharply differentiates the eosinophils and the basophils (9). We used, then, three methods for estimating the goitrogenic effect of sulfaguanidine: 1) weight of gland expressed as mg. per 100 gm. of body weight; 2) gross appearance; 3) microscopic appearance. Basal determinations were carried out in a Benedict calorimeter at a temperature of 27° to 28°C. on rats previously fasted for 20 hours. The activity of the animals was followed by means of a tambour connected with the movable bottom of the calorimeter. The surface area in square meters was estimated by the formula of Meeh, $S = 0.1 \times W^{\frac{2}{3}}$ with W in kilograms. Oxygen consumption was expressed as calories per square meter per 24 hours.

RESULTS

I. Prolonged Administration of Sulfaguanidine

The feeding of 2% sulfaguanidine to immature rats produces a 34% increase in thyroid weight over that of the controls in 48 hours. This rapid rate of increase in thyroid weight continues for the first 2 weeks on the diet, and thereafter begins to diminish somewhat, although it has not ceased at 8 weeks, when the increase equals 465%. Concomitant with the increase in thyroid size are histologic changes also evident in 48 hours, characterized by vacuolation and loss of colloid and general heightening of the epithelial cells. These histologic changes progress for 2 weeks, at which time the normally low cuboidal epithelium is completely transformed into columnar epithelium, and only occasional shreds of colloid remain in the follicles. This same histologic response has been observed after 100 days of drug administration. Studies on the rate of oxygen consumption in young animals fed the drug established a definite depression in the B.M.R. within 2 weeks (3).

In actively growing adult animals the increase in thyroid weight during a given time is not as marked as in immature animals. However, the histologic response is of the same intensity. As stated in the introduction a drop in B.M.R. coincides in time with the disappearance of colloid from the thyroid gland.

In still older animals who have passed the period of active growth the rates of histologic response and of weight increase are both reduced. Oxygen consumption studies have not been made on these animals.

Since we have observed such a prompt depression in the B.M.R., contrary to the results of Astwood et al. (5), this experiment was repeated on 4 sexually mature male rats. During 14 days of 2% sulfaguanidine feeding, the B.M.R. fell to −18% (Table 1). Extension of treatment to 28 days resulted in a further drop in the B.M.R. to −26% thus confirming our previous findings. The average thyroid weight of the 4 animals was 17.7 ± 0.75 mg. per 100 gm., a figure in keeping with previous results on growing adult rats. Histologic examination of the thyroids disclosed epithelial hyperplasia and the absence of all but remnants of colloid. Typical thyroidectomy cells were found in the anterior pituitary.

Having again obtained a depression in the B.M.R. in 2 weeks with sulfaguanidine, our attention was turned to the effect of prolonged administration when initiated at the time of weaning. From the results on animals of different ages reviewed above, any one of the following morphological responses seemed possible; first, the persistence of the marked hyperplasia produced in immature rats, second, regression of the gland to the condition found in older animals, third, exhaustion, atrophy and scarring, and fourth, neoplasia. The effect of prolonged sulfaguanidine feeding on the B.M.R. also presented a number of possibilities.

Thirty-four animals, 21 to 23 days of age and weighing 38 to 41 grams, were divided into two groups. Of this number, 26 were fed the stock diet containing 2% sulfaguanidine, and 8 were given the stock diet alone. After 120 days B.M.R. determinations were initiated on 6 of the drug-treated animals and 4 of the control animals and continued over a 3-week period. At the end of this time, 140 days, 16 of the animals receiving sulfaguanidine whose oxygen consumption had not been determined, were sacrificed along with 8 of the control animals including the 4 whose B.M.R.'s had been studied. Simultaneously, the drug was removed from the diet of the remaining 10 experimental animals in order to observe the effect of withdrawal after 2 and 4 week periods on the B.M.R. and the histology of the thyroid gland.

At 140 days the average B.M.R., based on three weekly determinations, of the six drug-treated animals was 483 ± 15.1 calories as contrasted to 691 ± 9.58 for 8 control animals (4 from this and 4 from a previous experiment). This difference represents a decrease of 30%. Indeed the B.M.R. of the animals fed sulfaguanidine was only 7% higher than the average obtained from 12 completely thyroidectomized rats of a similar age (Table 2).

Although the individual thyroid weights of the 16 experimental animals killed when the B.M.R.'s were determined on the 6 drug-fed animals varied within a wide range, it is evident from Table 3 that the gland was enlarged in every case. In 6 males the thyroid weight ranged from 17.4 to 55.4 mg. per 100 gm. body weight. In contrast the thyroid weights for 10 females varied from 21.1 to 85.0 mg. per 100 gm. body weight. The average thyroid weight for the males was 34 ± 5.8 and for the females, 57 ± 6.9. The difference between these averages when subjected to Fisher's "t test" gave the value of 2.54 indicating that the difference was highly significant. In contrast to this difference in size the gross appearance of the thyroid of both sexes was identical. The isthmus was thickened, the gland was enlarged in all directions, and striking hyperemia was invariably present.

TABLE 1

Decrease in the Basal Metabolic Rate Produced by the Addition of 2% Sulfaguanidine to the Diet of Young Adult Male Rats

ANIMAL*	NORMAL B.M.R.†	PERCENTAGE CHANGE IN B.M.R. AT VARIOUS DAYS AFTER TREATMENT		THYROID WEIGHT
		14 days	28 days	
				mg. per 100 gm.
1160 z	739	−17.0	−22.7	20.5
1161 z	749	−18.1	−32.8	15.3
1162 z	655	−22.9	−26.1	16.9
1163 z	682	−12.3	−23.7	17.9
Average.....	706 ±25.0	−17.6 ±2.10	−26.3 ±2.37	17.7 ±0.75

* Initial weight, 233–297 grams.

† Average of 3 readings taken at weekly intervals. B.M.R. expressed as calories per square meter per 24 hours.

TABLE 2

Effect of Feeding 2% Sulfaguanidine for 140 Days on the B.M.R. of Male Rats and Their Response to Its Withdrawal

NO. OF RATS	TREATMENT	B.M.R.*	PERCENTAGE CHANGE IN B.M.R. AT VARIOUS DAYS AFTER WITHDRAWAL		THYROID WEIGHT	THYROID HISTOLOGY†
			14 days	28 days		
					mg. per 100 gm.	
1	Drug 140 days‡	492	+20.6§		25.8	3–4+
1	" "	522	+33.5§		33.9	3–4+
1	" "	499	+28.9	+28.6	14.1	4+
1	" "	402	+32.9	+33.8	15.0	4+
1	" "	501	+28.1	+24.1	11.3	4+
1	" "	480	+30.7	+23.6	23.5	4+
6‡	Drug 140 days	483 ±15.1	+29.1 ±1.96	+27.5 ±2.46		
8‡	Stock diet 140 days	691.0 ±9.58			7.2 ±0.53	4+
12¶	Thyroidectomized, stock diet	450				

* Average of 3 readings taken at weekly intervals. B.M.R. expressed as calories per square meter per 24 hours.

† 3+ equals 75% colloid-flat and cuboidal epithelium.
4+ equals 100% colloid-flat and cuboidal epithelium.

‡ Average weight of rats at start of experiment 40 gm.

§ Sacrificed at 2 weeks.

¶ Male rats, 80–100 days of age at time of operation; B.M.R. determinations initiated 2 months after operation.

On histological examination, these glands presented a varied picture. In 3 animals whose thyroid weights were 17.4, 17.8 and 21.1 mg. per 100 gm. of body weight most of the follicles were packed with uniformly staining dense colloid, and lined with flat cuboidal epithelium. However, some groups of follicles were devoid of colloid and lined with columnar epithelium, the remaining evidence of the profound histologic response to sulfaguanidine at an earlier age. In striking contrast was the histology observed in 2 rats whose thyroid weights were 81.9 and 85.0 mg. per 100 gm. body weight. In only a few of the follicles were traces of colloid present. The acini were small, oval in shape, increased in number and lined with columnar epithelium.

TABLE 3

Effect of Feeding 2% Sulfaguanidine for 140 Days on the Thyroid of Rats and Their Response to Its Withdrawal

NO. OF RATS	SEX	AVERAGE INITIAL WEIGHT	AVERAGE FINAL WEIGHT	TREATMENT	AVERAGE THYROID WEIGHT	AVERAGE THYROID HISTOLOGY*
		gm.	*gm.*		*mg. per 100 gm.*	
6	Male	41	271	Drug 140 days	34.0 ±5.8	1+
10	Female	36	163	Drug 140 days	57.0 ±6.9	1+
4	Male	36	246	Drug 140 days, stock diet 14 days	21.8 ±5.1	3+
2	Female	36	149	Drug 140 days, stock diet 14 days	23.5 ±4.9	4+
4	Male	37	293	Drug 140 days, stock diet 28 days	16.0 ±2.57	4+
8	Male	39	332	Stock diet 140 days	7.2 ±0.53	4+

* 1+ equals 25% colloid—columnar epithelium; 3+ equals 75% colloid—flat and cuboidal epithelium; 4+ equals 100% colloid—flat and cuboidal epithelium. The average histological picture is given for each group.

The glands of 8 animals showed a more consistent and uniform response intermediate between those described above. Approximately 25% of the normal amount of the colloid was present in the follicles and the epithelium was either of the columnar or the high cuboidal type. In the 3 remaining glands detached epithelial cells containing pyknotic nuclei were found in some of the lumina. Such follicles contained shreds of colloid in some instances and in others none at all. This condition has also been described by Richter and Clisby and by Astwood et al. (4, 5). Despite the considerable variation in histologic response observed in these animals, no signs of atrophy or scarring were noted. The thyroid glands of the 8 control animals averaged 7.2 ± 0.53 mg. per 100 gm. body weight. The follicles were lined with cuboidal epithelium and packed with colloid.

In the 13 animals whose thyroid glands showed the most pronounced hyper-

plastic changes at 140 days, extreme histological alterations were also found in the anterior pituitary. The eosinophils were greatly reduced in number and degranulated, whereas the basophils were enlarged, vacuolated and hyalinized. The latter cells, the so-called thyroidectomy cells, were invariably present in large nests. Conversely, the pituitaries of the 3 rats whose thyroids had undergone regression, i.e. reaccumulation of colloid and return of the epithelium to the cuboidal form, contained the normal complement of granulated eosinophils. However, vacuolated and hyalinized basophils were still encountered although they were not present in the large groups seen in rats with extreme thyroid changes. These observations are in accord with previous reports on thyroidectomy changes in the anterior pituitary of rats fed sulfonamides and thiourea and its derivatives (3).

From the foregoing section of this paper several points emerge. First, that prolonged administration (140 days) of 2% sulfaguanidine produces no unique cellular reactions in the thyroid or pituitary that do not generally occur in shorter feeding periods. Second, that a variable degree of regression takes place from the maximum histological stimulus elicited with this level of drug in immature rats. Third, that the B.M.R. may be depressed by sulfaguanidine to thyroidectomy levels.

Sulfaguanidine was next withdrawn from the diet of 6 rats after 140 days of treatment at which time the average B.M.R. was 483 calories per square meter per 24 hours. At the end of 2 weeks the B.M.R. had risen to 682, an increase of 29.1 $\pm$ 1.96%. As was to be expected, since this level of oxygen consumption is within the normal range for our rats, no further increase occurred when the period of drug withdrawal was extended to 4 weeks.

The thyroids of 6 animals (including 2 of the above rats) sacrificed after 2 weeks on the stock diet alone were still enlarged although the hyperemia had almost completely disappeared. The glands weighed approximately 23 mg. per 100 gm. as compared to 7.2 mg. for animals of the same age who had never received sulfaguanidine. Nevertheless, this represented a considerable decline in thyroid weight in both males and females as a result of drug withdrawal (Table 3).

The histologic picture of the thyroids two weeks after the cessation of sulfonamide feeding was uniform. The acini were filled with colloid and lined with cuboidal epithelium. The follicles were uniform in size and all of them appeared to be smaller than those found in the periphery of the normal thyroid gland. The cellular alterations produced in the anterior pituitary by sulfaguanidine feeding had also disappeared at 2 weeks so that the glands contained an abundance of granulated eosinophils, and but infrequent hyalinized basophils.

Essentially the same picture was observed in the thyroid and pituitary glands of 4 rats sacrificed 28 days after the cessation of sulfaguanidine feeding. The thyroids had further decreased in weight although they were still more than twice as heavy as those taken from control animals (Table 3).

In 7 animals (3 males and 4 females) the feeding of 2% sulfaguanidine was started at weaning and continued for 220 days. The thyroids weighed 69.0 $\pm$

5.8 mg. per 100 gm. and there was no difference between the males and females. The absence of sex difference may be due to regressive changes, for histologically the follicles contained from 50 to 75% of the normal amount of colloid. While the majority of the follicles were lined with cells of the flat cuboidal type, a few still contained distinctly columnar cells. Shedding of the epithelial cells into the lumina was also evident in some of the follicles. Both granulated and degranulated eosinophils and vacuolated basophils were seen in the anterior pituitary.

II. Intermittent Feeding of Sulfaguanidine to Immature and Adult Rats

Having ascertained that the prolonged stimulation of thyroid cells effected by sulfaguanidine did not result in a riotous or carginogenic form of activity on the one hand, or in exhaustion atrophy on the other, our attention was turned to the effect on the thyroid of the intermittent feeding of two levels of sulfaguanidine. It seemed possible that one or the other of these reactions might be thus produced.

Immature Rats. Six groups of 6 to 8 immature rats each were fed the stock diet containing sulfaguanidine at a 2% level. A seventh group served as controls. Eight animals were fed the drug-containing diet for 2 weeks and allowed a similar period of recovery.

The procedure adopted for the other groups was as follows: Each group was subjected to 2 periods of drug administration and 2 periods of rest. At the end of the second rest period, one-half of the animals were sacrificed to determine the degree of thyroid regression, and the other half were subjected to an additional period of drug feeding to determine whether the glands were still capable of a third response. The duration of the drug periods was 2, 3 or 4 weeks, and that of the rest periods either 1 or 2 weeks.

The results of this experiment, tabulated in Table 4, show that the histology of the thyroid returns to normal, and that there is a reduction in size following drug withdrawal. The gland is still enlarged, however, after 2 weeks of rest. Administration of sulfaguanidine following 2 feeding and withdrawal episodes again elicits an increase in weight, loss of colloid, and epithelial hypertrophy. Throughout the experiment the female thyroids were larger than those of males.

The pituitary pattern was normal following all rest periods. However, the pituitaries of rats subjected to a third period of drug feeding contained vacuolated basophils and degranulated eosinophils. The most pronounced changes were seen in the animals receiving sulfaguanidine for periods of 4 weeks.

Adult Rats. A total of 24 animals a year or more of age and divided equally as to sex were employed for intermittent feeding studies. Eight of this number were fed a 2% level of drug in the diet, and another 8 were given a 3% level. The remainder served as controls. The general procedure was similar to that used for the immature animals. The period of rest throughout this experiment was the same, namely 2 weeks.

It will be seen in Table 4 that the response to intermittent therapy was similar, though not as striking, to that shown by the young animals. Furthermore, there

was no sex difference in thyroid weight. Although the thyroids of the adult animals fed the drug for a 2 week period were not so appreciably enlarged, histologic examination of the glands revealed a 50% loss of colloid and a heightening of epithelial cells. Withdrawal of the drug for 2 weeks resulted in the reversal of these changes. A second and third period of drug feeding again increased the weight of the thyroid gland and produced a marked histologic response.

In the adult animals fed drug for 2 weeks and then rested for 2 weeks, the pituitaries were essentially normal. In rats sacrificed after the third period of drug feeding, vacuolation of scattered basophils and the beginning degranulation of eosinophils was observed.

TABLE 4

The Effect of Alternate Feeding and Withdrawal of Sulfaguanidine on the Thyroid Gland of the Immature and Adult Rat

NO. OF RATS	INITIAL AGE	SEX	SULFAGUANIDINE	DRUG PERIOD	THYROID WEIGHT* AND HISTOLOGY† FOLLOWING TREATMENT					
					Controls	Drug	Rest‡	Drug	Rest‡	Drug
			%	wks.						
4	24 days§	♀	2	2	8.8¶	27.1¶			15.6	21.6
					(4+)	(0)			(4+)	(2+)
12	24 days§	♂	2	2	8.8¶	27.1¶	12.6		13.4	13.2
					(4+)	(0)	(4+)		(4+)	(2+)
6	24 days§	♀	2	3	8.8¶				22.4	28.0
					(4+)				(4+)	(1-2+)
6	24 days§	♂	2	3	8.8¶				15.1	18.6
					(4+)				(4+)	(1-2+)
6	24 days§	♀	2	4	8.8¶				20.1	39.4
					(4+)				(4+)	(1+)
6	24 days§	♂	2	4	8.8¶				14.3	23.8
					(4+)				(4+)	(1+)
8¶	1 year‖	♂ & ♀	2	2	7.8	8.3	6.3	15.6		15.0
					(4+)	(2+)	(4+)	(1-2+)		(2+)
8¶	1 year‖	♂ & ♀	3	2	7.8	13.1	9.7	12.9		12.9
					(4+)	(1-2+)	(2+)	(1-2+)		(2+)

* Mg per 100 gm body weight.

† (0) = no colloid, columnar epithelium; (1+) = 25% of colloid, columnar epithelium; (2+) = 50% of colloid, columnar and high cuboidal epithelium; (4+) = 100% of colloid, flat and cuboidal epithelium.

‡ One or 2 week periods.

§ Initial weight 32-52 gm.

¶ Data taken from previous publication (3).

‖ Initial weight 210-450 gm.

DISCUSSION

The depression in B.M.R. of sexually mature rats fed 2% sulfaguanidine for 2 weeks confirms our previous observations on this subject (3). The fact that Astwood et al. (5) obtained little or no change

in the rate of oxygen consumption during the first 7 to 14 days of treatment with sulfaguanidine may have been due to the method of administration they employed. The results of Astwood (7) with thiourea and thiouracil, and those of Williams and Bissell (8) with thiouracil in the successful treatment of hyperthyroidism in humans further suggest that our observations on the prompt depression of the B.M.R. in rats fed sulfonamides or thiourea are correct.

The consistent fall in the B.M.R. to thyroidectomy levels following the long term feeding of 2% sulfaguanidine is in harmony with the hypothesis previously advanced and discussed at length by ourselves (3) and by Astwood et al. (5) that the sulfonamides and thioureas inhibit thyroxin formation. However direct experimental evidence that the hormone output of the thyroid is reduced in vivo must be obtained before this theory is translated into fact. The failure of sulfaguanidine to inhibit the calorigenic activity of small doses of thyroxin in thyroidectomized rats (3), and of thiouracil to inhibit the response of myxedematous patients to desiccated thyroid (8) greatly favor this hypothesis, but do not prove it.

Since the withdrawal of sulfaguanidine resulted in a prompt rise in the B.M.R., it is evident that the mechanism involved, whatever its nature, is reversible. Previous results obtained by us in 2 rats that did not thus respond to the withdrawal of sulfaguanidine were either due to the older age of these animals or were in error.

Every experiment reported in this paper has abundantly confirmed the parallelism between the hypertrophy of the thyroid epithelium and the presence of thyroidectomy changes in the pituitary. Following the removal of sulfaguanidine from the diet the histology of the two glands returned to normal. Where the B.M.R. was measured its change was in the direction to be expected on the basis of histological evidence; evidence which when encountered clinically points to the exact opposite, the high B.M.R. of Graves disease. However, according to the theory previously advanced (3, 5), in the condition under discussion the inhibition of thyroxin formation and the concomitant reduction in oxygen consumption should be the factors determining the cellular changes in the thyroid and not visa versa. It is necessary to postulate that the pituitary with advancing age is less responsive to a deficiency of thyroid hormone and hence produces a lesser degree of thyroid hyperplasia and loss of colloid, or an alternate hypothesis

is that with increasing age the rat thyroid becomes less responsive to the pituitary thyrotropic hormone. In any case, the reduction of oxygen consumption effected by sulfonamides and thioureas can be inferred from the histological state of the thyroid only when the age of the animal is taken into consideration.

The withdrawal of sulfaguanidine after prolonged or intermittent treatment resulted in the reaccumulation of colloid and the transformation of the thyroid epithelium from the columnar to the normal cuboidal type within one week, although the weight of the gland had not returned to normal within 4 weeks. This may have been due to the presence of new follicles. It cannot be said, however, on the basis of present evidence that the enlargement would persist.

From the foregoing there appears little danger that the prolonged or repeated administration of sulfaguanidine or of other compounds possessing a similar thyroid-metabolic effect will produce either exhaustion atrophy or riotous activity in the thyroid of the normal animal. Unfortunately, no such conclusions can be drawn as yet with respect to their effect on labile or already hypo or hyperactive thyroid glands.

The absence of reports on thyroid alterations in humans treated with sulfonamides may indicate that in this respect they are unlike the rat, mouse, and the dog (3), and are susceptible only to the thioureas. The possibility remains that the disturbances in metabolism accompanying many diseases may offset such an action of sulfonamides in precisely those individuals most frequently receiving them.

Should cases of thyroid disturbance as a result of sulfonamide therapy ultimately come to light, the existing quantitative experimental data will form a basis for their rational management with thyroxin. However, the importance of this question goes far beyond the presence or absence of side reactions in sulfonamide therapy. It goes to the very root of the problems of thyroid function, and species specificity with respect to classes of organic compounds modifying thyroid function. Further, in view of the favorable results already reported by the Boston workers the presence or absence of such species specificity will determine to a large extent the chemical fields to be explored in search of the most suitable compound for the treatment or control of hyperthyroidism. In adult rats thiourea may be extremely toxic (10).

This paper re-emphasizes the previous conclusion that the younger

the animal the more pronounced the thyroid enlargement and hyperplasia. Indeed a level of sulfaguanidine producing a maximum hyperplastic response in immature rats may not maintain the intensity of this reaction as the animal grows older.

CONCLUSIONS

1. The administration of sulfaguanidine to young adult rats produced a sharp drop in the B.M.R., and a simultaneous hyperplasia and enlargement of the thyroid. Thyroidectomy cells appeared in the anterior pituitary.

2. Prolonged drug feeding, from the time of weaning, reduced the B.M.R. to thyroidectomy levels. Histologically, the thyroids varied from the normal to a condition of extreme hyperplasia. The average colloid content of the follicles was reduced by 75%. Thyroid enlargement in the female exceeded that in the male. Nests of thyroidectomy cells were present in the pituitary.

3. The withdrawal of drug from these animals resulted in a return of the B.M.R., and of thyroid and pituitary histology to normal. However, the thyroid glands were still enlarged at 4 weeks.

4. Intermittent sulfaguanidine feeding to immature and to adult rats produced neither a refractory nor a hypersensitive state with respect to thyroid stimulation. The gland became hyperplastic during each feeding period, and regressed during each withdrawal period.

ACKNOWLEDGEMENTS

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THE PATHOLOGY OF STAPHYLOCOCCUS ABSCESSES IN VITAMIN C-DEFICIENT GUINEA PIGS[1]

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Although much work has been done on resistance to infection in vitamin C-deficient animals, the exact nature of the local inflammatory reaction in that deficiency has not been reported. In general, experimental interest has centered on survival times of deficient and normal animals after injection of various amounts of *staphylococci*, *pneumococci*, tubercle bacilli, diphtheria toxin, etc. (1, 2, 3, 4, 5). Since scurvy itself is fatal in guinea pigs, interpretation of these experiments is difficult, and the relationship of vitamin C to resistance to infection is still in doubt. However, much is known about the role of vitamin C in wound healing. Since the detailed work of Wolbach and Howe in 1926 (6), the anatomical and physiological pathology of sterile incisions in stomach, skin, muscle and bone in vitamin C deficiency has been the object of much attention. The sequence of events of abnormal wound healing found in vitamin-C-deficient humans and animals has been carefully studied and described. Hunt (7) has pointed out the need for similar study of the exact nature of the local inflammatory reaction under well-controlled conditions. It was the object of this work to attempt such a study.

Methods. Adequate control of dietary experiments is especially necessary when resistance to infection is being studied. Paired feeding experiments have shown that the rapid weight loss of young guinea pigs on a vitamin C-free diet is not due to differences in food intake between deficient and control animals. However, food intake curves of such deficient animals show markedly diminished intake for the last 10 days or two weeks of life. Therefore, it is important to limit the intake of the controls so that, except for the inclusion of vitamin C, their diet will be entirely comparable to that of the deficient animals. For this reason, paired feeding was used in these experiments.

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The diet used was a stock diet of Rab-Ets, sold by the Domino Feeds Division, Chicago, Illinois. This diet comes in pellet form and contains adequate supplies of protein, carbohydrate, fat, minerals and all vitamins except vitamin C. This is attested by the fact that it supports normal growth and reproduction in guinea pigs when supplemented by either pure l-ascorbic acid or green vegetables. Given alone, the first clinical signs of vitamin C-deficiency appear in 250-gram guinea pigs in 8 to 10 days, with average survival times of 20 to 26 days for animals of that weight. Vitamin C supplements were given by pipette in the form of a solution of pure l-ascorbic acid in water. The solutions were made up from the crystalline preparation just before feeding. Fifteen mg. in 0.5 cc. of water was given daily to each animal, since estimations of the daily requirement for saturation of guinea pigs vary from 1.5 to 8.0 mg.

Animals were housed in individual wire mesh cages. They were fed from round pottery dishes four inches in diameter, with straight sides one inch high. Spilling from these dishes was minimal, and the weakened, deficient animals were able to get at their food. Fresh water was offered to all animals each day in the same type of dish.

Staphylococci were chosen to produce the abscesses because of the localized nature of the resultant lesions. Several strains of hemolytic *Staph. aureus* and several sizes of inoculum were tried in preliminary experiments. The strain used in final experiments came from a large carbuncle on a patient on the surgical wards. An inoculating dose of 1×10^8 organisms in 0.1 cc. of normal saline produced, quite consistently, a small localized abscess from which viable organisms could be obtained 10 to 14 days after infection. The inoculum was prepared by centrifuging an 18-hour broth culture of the organisms for 20 minutes at 3000 r.p.m. and resuspending the bacteria in an appropriate amount of normal saline.

EXPERIMENTAL

Animals and Diet. 250 to 300-gram guinea pigs of impure stock were used. All animals were fed the basal diet plus an l-ascorbic acid supplement by pipette for 2 weeks. Animals with satisfactory gain in weight for this period were chosen for the experiment. Animals were then paired by sex and weight, the members of each pair being

of the same sex and within fifteen grams of each other at the start. Paired feeding was started, and ascorbic acid was withdrawn from one member of each pair. Deficient animals were offered 40 grams of food per day. Their intake was weighed and the amount eaten was offered to the control member of the pair the following day.

Inoculation. Ten days after the withdrawal of ascorbic acid from one member of each pair, the animals were inoculated. Each animal was shaved and cleaned, and inoculated intracutaneously on the abdomen. An inoculum of 0.1 cc. was given on the right side, and 0.15 cc. on the left side. The suspension contained 1×10^9 organisms per cc.

Study of pathology.

Group I (6 pairs). The pairs in the first group of animals were permitted to live until the vitamin C-deficient member of any pair died. At that time the control animal was killed, and the lesions were fixed in 10 per cent formalin. No blood studies were made on these animals.

Group II (15 pairs). In this group, 3 or 4 pairs of animals were killed at intervals of 1, 3, 8, and 14 days after inoculation. Also, as controls, 3 unpaired animals on C-deficient diet were inoculated. Ascorbic acid was returned to the diet of these 3 controls after a period of deficiency of 23 days, 13 days after inoculation. They were killed 8 days after the return of vitamin C to their diet. Red blood cell counts, hemoglobin, hematocrit, and serum protein determinations were done on the blood of most of the animals in this group. Lesions of the abdominal wall were removed, fixed in Zenker's fixative, and imbedded in paraffin. Hematoxylin and eosin, Weigert-Gram bacterial stains and Mallory's connective tissue stains were made on sections of these lesions.

RESULTS

Clinical Manifestations

Deficient animals continued to gain after the withdrawal of ascorbic acid, usually reaching their maximum weight 8 days after withdrawal. Control animals continued to gain for an average of 13 days after which they stayed constant or lost weight. Average losses of weight from the maximum of deficient and control animals at the time they

were killed are shown in Table I. Deficient animals in both groups developed typical signs of scurvy. Among these were enlarged joints, refusal or inability to move the hind legs separately, emaciation, anorexia, ruffled, lusterless hair, and the characteristic hunched-up posture. Three animals had grossly bloody stools. The animals in Group I which were permitted to die of scurvy showed great emaciation and weakness before death. They passed rapidly from a state of being able to take small amounts of food and water into a moribund state in which they lay helpless on their sides with rapid, shallow respirations, barely responding to painful stimuli. Control animals appeared normal throughout. The 3 unpaired deficient controls had developed typical signs of scurvy by the time vitamin C was resumed. They continued to lose weight 4 to 5 days after vitamin C supplements

TABLE I

Average Loss of Weight from the Maximum of Deficient and Control Animals

DAYS AFTER WITHDRAWAL OF VITAMIN C	WEIGHT LOSS FROM MAXIMUM (GRAMS)	
	Deficient animals	Control animals
12 (average of 3 pairs)	54	20
15 (average of 3 pairs)	72	15
18 (average of 3 pairs)	58	22
24 (average of 4 pairs)	90	33

were started. When sacrificed, the gait and posture of scurvy had disappeared except for slight residual stiffness of gait in one animal.

Appearance of lesions. There was no difference between the lesions of deficient and control animals in the degree of erythema and edema which developed immediately after injection. The lesions varied in size from 1.0 to 2.5 cm. in diameter, and were never raised more than 0.5 cm. from the skin surface. There was apparently no consistent difference in size of lesions among the control and deficient animals. In 4 to 5 days the lesions of the control animals rounded up and became button-like. This appearance suggested that the lesions were walled off, with necrotic caseous centers. The lesions of the deficient animals remained soft and of cheesy consistency, with no evidence of demarcation from the surrounding tissue.

Blood studies. Table II shows the results of blood and serum

determinations on the animals indicated. Serum protein determinations in the animals which had received a deficient diet from 18 to 24

TABLE II

Blood Studies on Paired Deficient and Control Animals

PAIRS	DAYS ON DEF. DIET	DAYS AFTER INOCULATION	HT.	HGB.	R.B.C.	M.C.V.	M.C.H.	SERUM PROTEINS	AV. FOOD INTAKE PER DAY
			%	*gms.*	*mil.*			*gms.*	*gms.*
Deficient 77	13	3	43.0	15.0				4.60	25.0
Control 78			40.5	12.6				4.00	
Deficient 51			46.0	14.5				7.00	18.6
Control 76			45.0	14.8				5.50	
Deficient 61			42.0	14.4				5.00	20.2
Control 62			47.0	13.6				5.50	
Deficient 65	18	8	33.0	11.0	4.09	79	27		18.2
Control 66			41.0	15.3	5.03	81	30	5.40	
Deficient 67			36.0	13.0	4.30	84	30	3.50	25.2
Control 68			40.0	14.9	4.96	80	30	4.60	
Deficient 79			38.0	14.3	4.62	83	31	3.50	33.0
Control 80			48.0	16.6	5.47	87	30	6.50	
Deficient 55	24	14	23.5	7.9	3.13	75	25	3.50	18.4
Control 56			45.0	16.0	5.52	81	29	5.50	
Deficient 57			38.0	12.3	4.92	77	25	4.40	21.4
Control 58			43.5	15.0	5.19	84	29	6.50	
Deficient 71			22.0	7.5	3.13	70	23	2.80	15.0
Control 72			45.0	15.0	5.50	81	27	6.30	
Deficient 88	24	14		14.0					
Deficient 89				14.4					
Deficient 90				13.7					
88	24 deficient	22	43.0	14.4	5.03	85	29	4.50	
89	+ 8 vit. C		47.5	17.0	6.70	71	25	5.50	
90			46.5	16.0	6.37	73	25	5.70	

days were low when compared to their controls, which had been given the same quantity of food. The same animals showed a variable

degree of anemia, as seen in hemoglobin and hematocrit determinations. The 3 animals given vitamin C supplements after 24 days on the basal diet alone showed no anemia.

Gross Pathology

Scurvy. At autopsy, typical scorbutic changes were observed in the deficient animals. Beading of the costochondral junctions was found even in those animals that had received the deficient diet for only 12 days. An irregular border of the marrow cartilage junction could be seen if the ribs were held up to the light. Hemorrhagic enlargement of elbow and knee joints were uniformly present. Subcutaneous hemorrhages and some subcutaneous edema were present in animals which were killed or died after 20 or more days on the deficient diet. Submucous hemorrhages in the bowel were present in 4 animals and grossly evident hemorrhages in the adrenal in one animal (#53).

Lesions. When the lesions of the abdominal wall were removed with a wide zone of surrounding tissue, it was found that the lesions of the deficient animals were poorly defined. The older lesions in the deficient animals showed grossly visible hemorrhage when viewed from the peritoneal surface. Of the 6 earlier lesions, 1 and 3 days after inoculation, only one showed grossly evident hemorrhage.

Microscopic pathology of lesions

Twenty-four hours after inoculation.

Three deficient animals. Cellular infiltration was diffuse throughout the subcutaneous tissue and the first muscle layer. Edema and a few localized areas of hemorrhage were present. The cellular reaction consisted predominantly of polymorphonuclear cells, with only a few round cells at the periphery in a ratio of approximately 10 polymorphonuclears to 1 mononuclear cell. Weigert-Gram bacterial stains showed many viable-looking organisms scattered through the area of reaction, many of which were intracellular. Broken strands of collagen from the subcutaneous tissue were present. Few capillaries were in evidence.

Three paired controls. There was no difference between the control and deficient groups in size of reaction, degree of edema, amount of

hemorrhage, or presence of capillaries. There was no important difference in the histological picture at the center of the reaction where, in both deficient and control animals, the predominant cells were polymorphonuclear. However, at the periphery of the reaction many more round cells were seen in the control animals. Here the ratio of polymorphonuclear leukocytes to round cells was approximately 1 to 1.

Seventy-two hours after inoculation.

Three deficient animals. The center of the abscess was a circumscribed, rounded area of necrotic cells. The border of the abscess was relatively acellular, but macrophages were more abundant than in the 24-hour sections. The ratio of polymorphonuclear leukocytes to round cells in the peripheral reaction was now 1 to 1. Only a few cells at the peripheral part of the reaction could definitely be identified as fibroblasts, having flattened nuclei with small amounts of cytoplasm projecting as points at the ends of the nuclei. Many dilated capillaries were seen at the border of the abscess proper. There were no gross areas of hemorrhage, but free red blood cells were scattered around the areas where capillaries were in evidence. There were thick bands of material which took the Mallory connective tissue stain around the necrotic center of the abscess. These were not wavy or fibrillar in appearance and were in no evident relation to any of the cellular components or capillaries. In hematoxylin and eosin-stained sections, the material had a hyaline-like appearance. Actively proliferating bacteria were present in the defined, necrotic region, both intra- and extracellularly, often in clumps. Few bacteria were seen in the surrounding reaction.

Three paired controls. Two of the 3 animals showed a localized necrotic focus of phagocytizing polymorphonuclears and proliferating bacteria. At the border of this defined area in the peripheral reaction, macrophages were much more in evidence than in the deficient group. In this zone only an occasional polymorphonuclear cell was seen, macrophages and other round cells being present in great abundance. This contrasted with the relatively large number of polymorphonuclear cells seen in the deficient animals in the corresponding part of the reaction. Many capillaries were seen at the border of the necrotic focus. There were no areas of free red blood cells or gross hemor-

rhage. Actively proliferating bacteria were present, confined to the defined necrotic area, as in the deficient animals.

Eight days after inoculation.

Three deficient animals. Two of 3 animals showed localized necrotic foci with a wide peripheral zone of disorderly and loosely-arranged connective tissue. In the third animal only a wide zone of similar connective tissue reaction between the skin and first muscle layer and again between the first two muscle layers was seen. In these animals the breadth of the peripheral connective tissue reaction was out of all proportion to the size of the necrotic focus. It consisted of irregularly arranged, loosely-spaced, stellate connective tissue cells showing little cytoplasm. The interval between cells was taken up by a very faintly and unevenly stained material. Nearer the center of the abscess there were lakes and columns of hyaline-like material which stained heavily blue with Mallory stain. Many pigment-containing macrophages were present at the border of the necrotic focus and many free red cells were scattered through the same area (Plate Ia). Numerous small, defective-looking capillaries were seen in the peripheral reaction. In some the lumina were distended, but empty. In others the empty lumen was almost occluded by hypertrophic endothelial nuclei. Bacterial stains showed viable looking bacteria.

Three paired controls. Two of 3 animals showed necrotic foci with a peripheral zone of compact, radially arranged, connective tissue. One animal showed only a small focus of granulation tissue without stainable bacteria. The connective tissue reaction was strikingly less wide than in the deficient animals. Many more macrophages were seen at the border of the necrotic focus. The connective tissue taking the Mallory stain consisted of dense wavy strands of normal-looking collagen with a definite fibrillar structure (Plate Ib). Invading the necrotic focus were tongues of granulation tissue in which capillaries, macrophages, and fibroblasts could be seen. No free red cells or pigment-containing macrophages were present. Many filled, normal capillaries could be seen in the peripheral zone.

Fourteen days after inoculation.

Four deficient animals. Two animals showed grossly undefined, necrotic foci consisting of macrophages containing bacterial and cel-

lular debris, among which there were much hemorrhage, an occasional fibroblast, but no normal capillaries. These two, and a third with no necrotic focus, showed a wide zone of abnormal connective tissue reaction. In only one animal were viable-looking bacteria present. These were all intracellular in macrophages scattered throughout the necrotic focus and in the peripheral connective tissue. Many free red cells and some grossly evident hemorrhages were present in all animals. Pigment-containing macrophages were plentiful, especially near the necrotic foci (Plate IIa). The connective tissue was similar to that in the 8-day sections described above, but it was even more loosely arranged and edematous. Near the necrotic foci the same columns and lumps of heavily stained material were seen (Plate IVb). Fewer defective capillaries were seen than in the 8-day sections.

Four paired controls. Two animals showed definite necrotic foci, both sharply defined and both surrounded by compact, narrow, radially arranged zones of typical connective tissue reaction (PlateIIb). Tongues of ingrowing granulation tissue were present. Viable-looking bacteria were seen in only one section. Dilated capillaries were prominent.

Pairs of animals of Group I died or were killed 10, 11, 12, 16, and 18 days after inoculation. The pathology of the earlier lesions was similar to those already described in Group II, and the later ones indicated a continuation of processes which were observed in the lesions of Group II. At 16 days the abscess of the scorbutic animal had a medium-sized necrotic center with a huge connective tissue reaction extending through both muscle layers. Free red cells and many pigment-containing phagocytes were seen at the edge of the necrotic center. The peripheral reaction consisted of numerous fibroblasts thickly grouped and arranged in disorderly fashion with scarce, widely-dispersed, diffuse bands and columns of poorly-staining intercellular matrix. The control animal showed a medium-sized, sharply defined abscess with a small reaction around it. Fibroblasts were plentiful and radially arranged, with a meshwork of connecting bands of collagen, walling off the abscess in an orderly way.

Eighteen days after inoculation.

The two scorbutic animals had small areas of necrotic tissue with huge reactions around them, which extended through and destroyed

both muscle layers (Plate IIIa). Near the center of the abscess there were many free red blood cells and pigment-laden macrophages. Stellate cells with large nuclei were a prominent part of the reaction. Around the necrotic area there were dense clumps and lakes of amorphous, poorly-staining intercellular material. Nearer the periphery of the reaction the fibroblasts were more densely packed with only a few strands of collagenous material between them (Plate IVa). Capillaries were scarce and those few that were present consisted of closed columns of cells.

In the control animals the reaction was strikingly less wide in comparison with the size of the necrotic focus (Plate IIIb). Elongated fibroblasts were plentiful and were oriented around the abscess. Though the abscess was orderly and well-walled off, the collagen was in strands with no definite fibrillar appearance. It was possible that these animals had a minimal vitamin C deficiency. There were many normal-looking capillaries present, and in general the healing process appeared normal.

Treated animals. Two of the 3 animals that were on a vitamin C-deficient diet for 24 days, followed by 8 days of vitamin C supplement, had medium-sized necrotic foci with large reactions penetrating the first and second muscle layers, with some destruction of muscle. The third had a small flat focus of debris with muscle layers intact. A few polymorphonuclear leukocytes were seen in all. There was some hemorrhage, and many pigment-containing macrophages were seen at the border of the abscesses. The connective tissue reaction was wide in proportion to the size of the necrotic focus, but it was more orderly than that of the unsupplemented animals. There was an orderly zone of thin, wavy, fibrillar connective tissue strands. This normal-looking connective tissue was laid down in circular fashion throughout the wide areas of previous reaction. The nuclei of the fibroblasts had small clear zones, as though retraction of the matrix had taken place. The nuclei were no longer stellate, but flat and elongated.

Summary of Pathological Findings

In the deficient animals the initial response to the injected bacteria was a prompt outpouring of leukocytes and serum from the blood vessels. Free red blood cells were also present, although there was no

gross hemorrhage. The number of round cells initially was markedly less than in the control animals. Active phagocytosis of bacteria took place. At the third day macrophages and other round cells were still small in number, as compared with the control animals. Bacterial proliferation continued, but not more actively than in the controls, and phagocytosis continued. Defective capillaries and free red blood cells were evident. From the eighth to the fourteenth day, gross hemorrhage around the necrotic focus appeared. Pigment-laden macrophages surrounded the hemorrhagic zone and merged into a zone of defective connective tissue reaction. This zone widened with the passage of time, and its width was far greater than the orderly, concentrically-arranged fibroblasts and collagen fibrils of the control animals. This defective connective tissue corresponded microscopically very closely with the tissue described by Wolbach in the reaction to sterile incisions in muscle tissue (8). In some areas in the deficient animals, the whorls of cells were so thick and disorderly that the connective tissue reaction resembled a tumor.

No normal collagen fibers were seen at any stage in the deficient animals. From the third day irregular columns and lakes of a dense, more or less homogeneous substance resembling hyaline, and staining well with Mallory stain, was seen around the focus of phagocytizing and necrotic white blood cells. This material corresponded to that mentioned by Aschoff and Koch as occurring at the site of fractures at the costochondral junction of scorbutic humans (9). It was described by Hojer, who thought it was bone (10), and by Hess, who called it "protective fibrin" (11). Wolbach believed that it was an abnormal liquid product of connective tissue cells which in scurvy failed to gel (8).

DISCUSSION

The local inflammatory reaction in vitamin C-deficient guinea pigs was quite abnormal. Grossly, the lesion failed to round up. In the normal control animals, the abscess became hard and button-like, whereas in the scorbutic animals, the lesion remained soft and undemarcated. Arey believes that this rounding-up process is "surely due in considerable measure to the production and subsequent shortening of connective tissue fibrils" (12). The failure of this process in the deficient animals seemed to be due to continued hemorrhage into the

area, to defective fibril formation and resultant lack of contracture, and to the disorderly arrangement and lack of polarization in scorbutic connective tissue.

The continuation of fibroblastic proliferation in the lesions of deficient animals was difficult to explain. This had been observed also in the healing of sterile incisions. Hunt, working with partially deficient guinea pigs, observed that new fibroblasts began to appear on the third post-operative day in both deficient and control animals and multiplied to about the same extent (7). In the control animals, this proliferation ceased on about the fourteenth day, when the scar was consolidating, whereas in the sub-scurvy animals, it continued long beyond that time. He concluded that fibroblasts will continue to multiply so long as there is need of the formation of scar tissue, and, if this formation is delayed, cellular proliferation will be prolonged. It seems likely that the continued growth of fibroblasts was a secondary effect, due perhaps to maintenance of food supply or stimulation by products released from injured cells, rather than to ascorbic acid deficiency itself. Evidence for this view was found in an experiment by Follis in which one leg of the scorbutic guinea pigs was immobilized by a cast. In the immobilized leg there were no fractures, no hemorrhage, and no proliferation of connective tissue cells (13).

The low level of serum proteins observed in these animals may be of some significance. However, Thompson et al. found that the effect of hypoproteinemia on wound-healing in dogs was a marked delay and depression of fibroblastic proliferation (14). A slighter degree of hypoproteinemia may affect the production of collagen without inhibiting the proliferation of the cells; or there may be a fall in one fraction of the serum proteins which affects the production of intercellular substances—whether bone, collagen, or dentine—without producing the effects of general hypoproteinemia. It would be valuable to study the electrophoretic patterns of the serum proteins of animals wholly or partially deficient in vitamin C, and of paired control animals.

SUMMARY

The pathological processes involved in the healing of staphylococcal abscesses in scorbutic guinea pigs were found to be quite different from

those of normal paired control animals. In the initial reaction surrounding the inoculation the appearance of macrophages was delayed and the total number was reduced. The polymorphonuclear reaction was prompt and proportionate, and phagocytosis by these cells and by the macrophages was normal. However, more bacteria appeared to be extracellular in the abscesses 1, 3, and 7 days after inoculation in the scorbutic animals than in the controls. The connective tissue reaction was similar to that described by other workers in the healing of sterile incisions. Proliferation of fibroblasts continued after it had ceased in the control animals, and no normal collagen was formed. The connective tissue and the abnormal, hyaline-like intercellular substance which was formed, were disorderly in arrangement and the necrotic centers of the abscesses were not walled off. Blood studies showed anemia and low serum proteins in the scorbutic animals.

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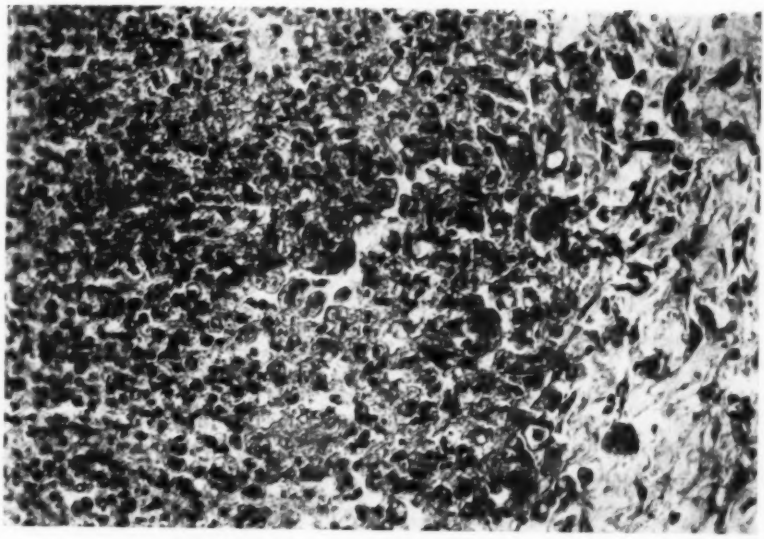
Plate Ia. #79; scorbutic diet 18 days. Edge of abscess 8 days after inoculation. The necrotic focus is surrounded by a zone of hemorrhage and pigment-containing macrophages. Outside of this there is an area of abnormal connective tissue. Hematoxylin and eosin (× 450).

Plate Ib. #80; control. Edge of abscess 8 days after inoculation. Necrotic focus is walled off by normal connective tissue, concentrically arranged fibroblasts and wavy, fibrillar collagen. Hematoxylin and eosin (× 450).



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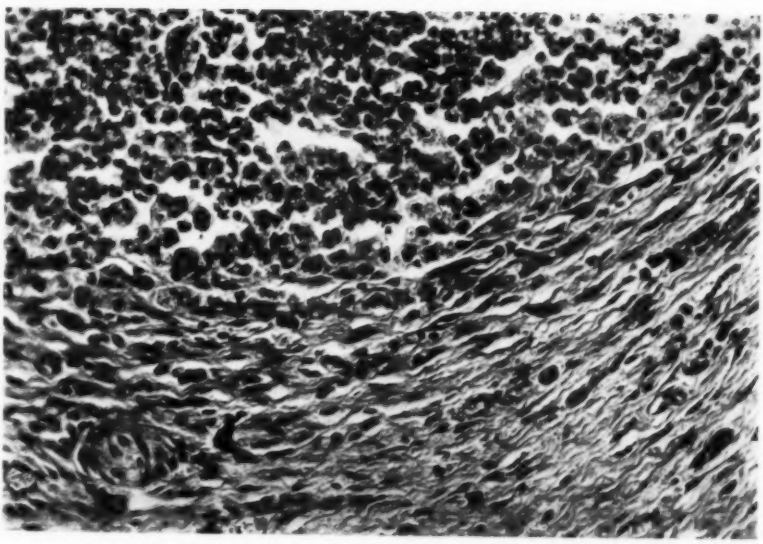

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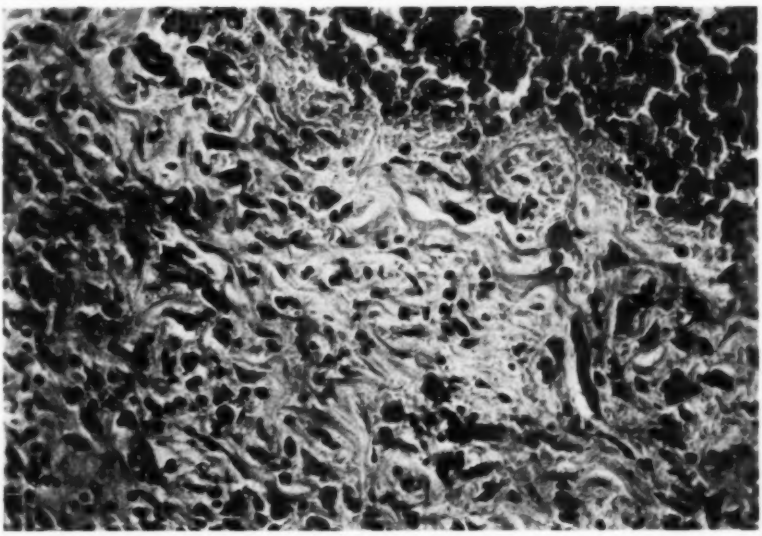
PLATE IIa. #71; scorbutic diet 24 days. Abscess 14 days after inoculation. Pigment-containing macrophages and thick, wavy bands of intercellular material. Hematoxylin and eosin (× 450).

PLATE IIb. #72; control. Edge of abscess 14 days after inoculation. Normal granulation tissue. Hematoxylin and eosin (× 450).



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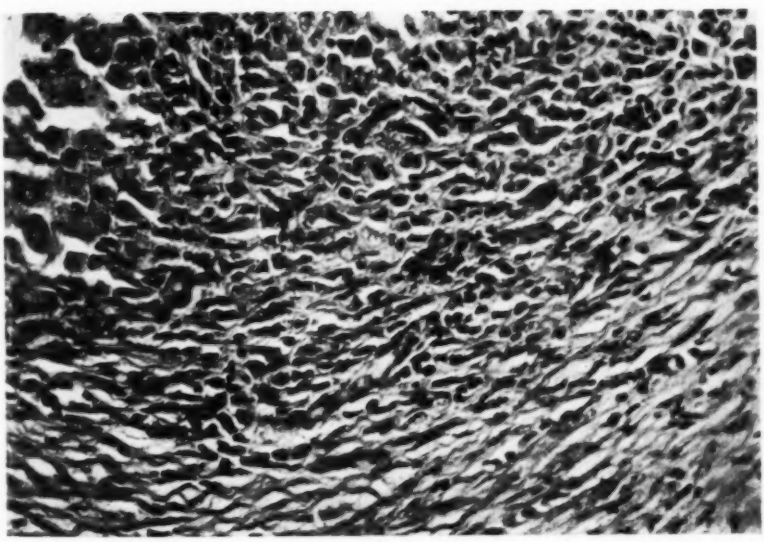
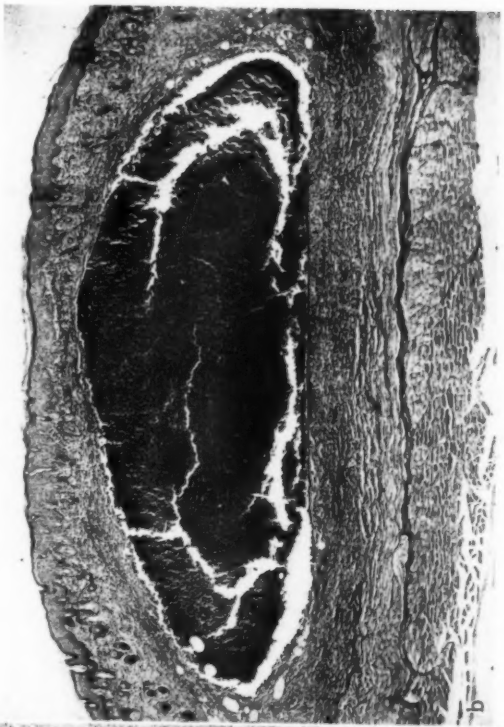


b

PLATE IIIa. #43; scorbutic diet 28 days. Abscess 18 days after inoculation. Small abscess with tremendously wide, disorderly connective tissue reaction and destruction of muscle layers. Hematoxylin and eosin (× 36).

PLATE IIIb. #44; control. Abscess 18 days after inoculation. Circumscribed abscess with narrow, orderly zone of normal connective tissue. Hematoxylin and eosin (× 36).



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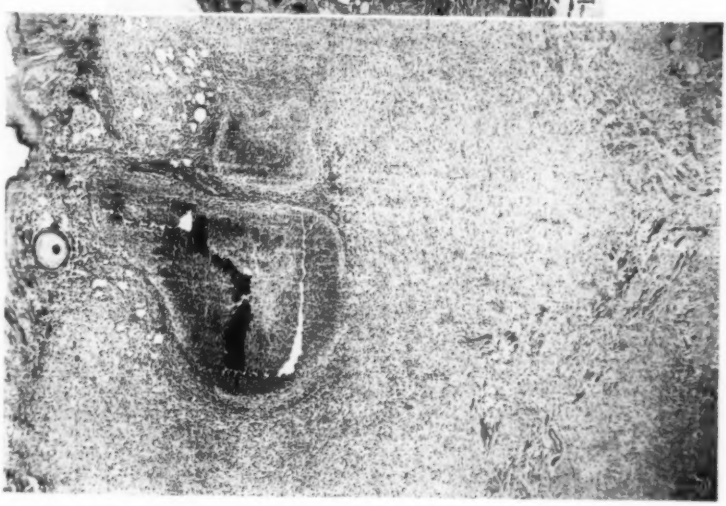
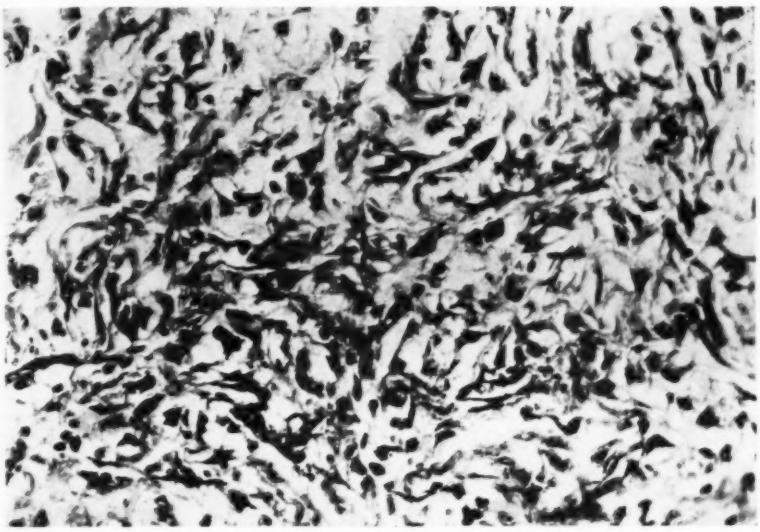


a

PLATE IVa. #43; scorbutic diet 28 days. Cellular reaction 18 days after inoculation. Cells are growing in tumor-like disorder with sparse, poorly staining intercellular material. Hematoxylin and eosin (× 450).

PLATE IVb. #71; scorbutic diet 24 days. Reaction 14 days after inoculation. Lakes and columns of hyaline-like intercellular material. Hematoxylin and eosin (× 450).



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b

A PRELIMINARY REPORT ON A METHOD FOR LENGTHENING THE EFFECT OF A SYMPATHETIC NERVE BLOCK

FERDINAND C. LEE, DAVID I. MACHT AND ROSS Z. PIERPONT

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The purpose of this preliminary communication is to report a method for lengthening the effect of a sympathetic nerve block. Ordinarily, when the lumbar sympathetic chain is blocked by a paravertebral injection of novocain or procain, the resultant increase in the temperature of the skin of the foot as well as the concomitant dryness of the skin of the leg rarely lasts more than four hours; in old, arteriosclerotic individuals the effect may occasionally persist for twenty-four hours. Among a number of substances used in casting about for a method to prolong the effect of the sympathetic nerve block, mono-brom-hydroxybenzyl alcohol was tried. Since this drug is only slightly soluble in water, peanut oil was used as a vehicle. Sterile ampoules of ten cubic centimeters of peanut oil containing four percent mono-brom-hydroxybenzyl alcohol were prepared.[1] Paravertebral injections were then made in the usual fashion, ten cubic centimeters of peanut oil containing the bromsalizol were injected in the region of the second lumbar segment and in addition an equal dose was given either in the first or third lumbar segments. The temperature of the skin of the foot did not increase until three hours later, but then the foot became warm and remained warm for five consecutive days, at the end of which time the temperature gradually fell until the effect had passed off entirely eight days after the injection had been made. Altogether twenty-six patients have received the injections. Of these twenty-four reacted as described above. Of the remaining two, the total effect lasted only four days in one case, and in the other no effect at all was observed. In its clinical application, all in old arteriosclerotic patients, it has: (1) cleared up cellulitis of the foot, (2) rapidly lessened the size of an intractable ulcer at the site of an amputation of three toes (3) cleared up an arterial thrombosis in the lower part of the leg and (4) probably

[1] This material under the name of Bromsalizol was supplied by Dr. Macht through the courtesy of Hynson, Westcott & Dunning, Inc.

helped in producing an unusually early well healed supracondylar amputation stump. Although the method was developed primarily to avoid the almost daily injections for the cure of thrombophlebitis, it is reasonable to believe that it may prove helpful in any case requiring improved circulation in the extremities; it may thus be of service in the present war. To what extent it can serve as a substitute for sympathetic ganglionectomy remains to be determined.

THE NUTRITIONAL REQUIREMENTS IN INANITION

I. Observations on the Ability of Single Foodstuffs to Prolong Survival[1,2]

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The problem of inanition is one which possesses great interest for the clinician as well as for the physiologist. Both in medical and surgical diseases of the gastrointestinal tract situations are frequently encountered in which the digestive organs are unable to handle adequate amounts of food and some degree of inanition results; if the disturbance is sufficiently prolonged death from inanition may ensue. To some extent this situation can be met by parenteral feeding, but up to the present time parenteral feeding has been limited in scope. Of the thirty or more foodstuffs which are now recognized as necessary to maintain life only three—water, sodium chloride, and glucose—are used routinely for parenteral administration. Preparations of amino acids and of B vitamins suitable for intravenous use have been introduced only recently and have not come into general use. Sporadic attempts have been made to give fat, minerals other than NaCl and other vitamins but such procedures cannot be said to have passed beyond the experimental stage.

The development of parenteral therapy has been hindered by difficulties in administering one or another type of foodstuff; a further retarding factor has been the *lack of fundamental knowledge of nutritional requirements in inanition*. We possess considerable information about the nutritional requirements for growth and maintenance; this cannot, however, be taken as a guide to requirements in inanition states, for the reserves of particular foodstuffs may differ widely and the stores of each are not necessarily exhausted at the same rate. Under such emergency conditions the need for particular foodstuffs

[1] The material in this paper was presented before the American Pediatric Society at Sky Top, Pa., on April 27, 1939. An abstract was published in Am. J. Dis. Child., **58**: 669, 1939.

[2] Aided by a grant from Mead Johnson and Company for the study of nutrition.

may be acute whereas the reserves of others are in no immediate danger of depletion. If it were possible to supply ample quantities of all foodstuffs the need for a more accurate knowledge of these conditions would not exist, but as is well known it is frequently impossible to do this. Deprivation of particular foodstuffs is often followed by lack of tolerance. The most familiar example of this kind is "starvation diabetes," but the ability to utilize fat and protein are likewise diminished when these foodstuffs are withdrawn from the diet and evidence has recently been obtained that the same principle holds for some of the B vitamins. When the quantity of utilizable food is limited it becomes important that the feeding should be adapted to the most urgent needs of the body, whatever they may be.

It is hardly to be expected that deficiencies developing in inanition states will conform to the patterns seen when unbalanced diets are consumed *ad libitum*. The possibility that under these conditions deficiencies may develop of types hitherto unrecognized must be seriously considered. The cause of death in inanition may well be due to the development of some one particular deficiency, as yet unidentified. Many theories as to the immediate cause of inanition death have been advanced, but the attempts to throw light on this phenomenon have done little more than throw into relief the fact that death from inanition is not due to lack of calories. Starved animals invariably contain some subcutaneous fat and a considerable quantity of dispensable tissue protein that could have been used to supply energy had not some other mechanism failed.

The present report deals with experiments in rats designed to throw light on the nutritional requirements in inanition. It was felt that under given conditions of dietary restriction—complete or partial—the ability of single foodstuffs to prolong survival or to produce clinical benefit would indicate a need for such foodstuff. Experiments of this kind are open to the criticism that beneficial effects secured from one foodstuff rather than another may not represent a superior nutritional value or a more acute need but may be due rather to some greater appeal to the appetite of the animal, resulting in increased ingestion. The appetite of the rat is certainly not an invariable criterion of the value of the food to him. As is well known he can be tricked into taking a fatal dose of poison and it is possible to disguise a necessary

food constituent with material of such evil taste or smell that he will not ingest it. But, such conditions are exceptions. As a general rule, these animals exhibit an extraordinary skill in making beneficial dietary selections, a fact that has been amply demonstrated by a number of workers (1), notably by Richter and his collaborators (2). In many deficiency states there develops an increased appetite for the missing factor or factors. Richter has shown (3) that rats offered a great variety of pure foodstuffs would not only select quantities suitable for optimal growth, but would choose their diets so efficiently that this optimum growth was obtained with an economy of 18.7 per cent in the caloric intake as compared with a standard McCollum diet. There is, therefore, justification for assuming that in inanition states the rat will eat what he can use, and that his survival will indicate with a considerable degree of accuracy his need of particular foods made available to him.

The questions which prompted the present work were the following: (1) the relative merits of carbohydrate, fat and protein in inanition states, (2) the merits of particular carbohydrates, fats and proteins, and (3) the extent to which various accessory factors become depleted in inanition. Although conclusive answers to these various questions have not been obtained and the work planned has not been carried to completion it is felt that the results obtained so far are of sufficient interest to report in detail.

Some preliminary orientation experiments, carried out in collaboration with Drs. Richter and Barelare (3), indicated that marked differences existed in the ability of particular foodstuffs to prolong survival. A subsequent report by Richter (4) has extended these observations to include a study of seven carbohydrates, nine fats and ten proteins. In these experiments the number of animals in each group was small (four in most instances) and the individual variation was considerable. It was felt that more conclusive results could be obtained by using larger numbers of animals and by employing smaller rats whose response is somewhat more uniform.

EXPERIMENTAL PROCEDURE

Our studies were carried out on male rats from a mixed colony of albino and hooded Norwegian rats. The animals were placed on the

experimental diet of single foodstuffs at the age of 30–35 days, their weight at that time being between 63 and 68 grams. They were kept in individual cages designed to prevent coprophagy. Food and water intake were allowed *ad libitum*, the quantities taken being accurately recorded. Weights were taken daily. As a rule 15 to 25 animals were employed for each group, smaller numbers being used in a few instances, as indicated. Some observations were made on larger animals for purposes of comparison.

RESULTS

I. A Comparison of the Ability of Carbohydrate, Fat and Protein to Prolong Survival

Glucose, olive oil and vitamin-free casein were chosen as representative of each group because of their satisfactory showing in the preliminary experiments referred to above. The survival time of small animals (63–68 grams) on the three foods mentioned is shown in Table 1. Rats on glucose survived an average of twenty-seven days, a period identical with that of the animals receiving olive oil. Somewhat surprising was the relatively short survival on casein (average 8.5 days). We were somewhat loath to conclude that protein was an inferior food in this situation and sought for some other explanation of this finding. This was provided by the study of the food intake on the different foodstuffs. It was noted that rats suddenly shifted from a mixed diet to a diet of pure fat or carbohydrate would seldom consume their entire caloric requirement from the single foodstuff on the first or even the second day of the new diet. Usually two or three days were required in the case of carbohydrate and about a day in the case of fat, during which time the food intake rose to a maximum and fairly adequate intake. This maximum was, however, maintained for a short time only; usually by the fifth day the food intake began to decline, decreasing steadily until the death of the animal. In the case of the animals shifted to protein alone the sharp rise in food intake failed to occur; instead, a very gradual rise was noted which in only one or two animals approached an adequate caloric intake before it was superseded by the inevitable progressive failure of appetite. Apparently, the adaptation to a high protein intake is a more difficult matter for the animal to accomplish than the adaptation to a high fat

or carbohydrate diet. It has been clearly demonstrated (5) that increasing the protein intake leads to notable hypertrophy of the liver and kidney, the new tissue being apparently needed to accomplish the additional deamination and urea excretion required by the protein increment. It seemed likely that these small rats were unable to accomplish the necessary tissue synthesis before the noxious effects of inanition developed; they were thus unable to utilize, and hence failed to consume, as much food in the early stages of inanition as did the carbohydrate- and fat-fed animals.

In order to test out this possibility, two sets of experiments were carried out. Single food experiments were carried out on larger ani-

TABLE 1

Survival Time of Small Rats on Single Foodstuffs

FOOD	SURVIVAL TIME OF INDIVIDUAL RATS	AVERAGE SURVIVAL
	days	*days*
Glucose	10, 11, 21, 23, 24, 24, 25, 25, 26, 26, 26, 27, 27, 27, 28, 28, 28, 28, 28, 28, 29, 29, 29, 29, 29, 29, 31, 31, 31, 31, 31, 32, 32, 36, 36.	27.3
Olive oil	14, 19, 21, 22, 22, 23, 23, 23, 23, 24, 24, 25, 25, 25, 26, 26, 26, 27, 27, 27, 28, 28, 28, 28, 30, 31, 31, 31, 32, 32, 33, 34, 38, 41.	27.1
Casein	3, 4, 4, 4, 4, 5, 5, 5, 5, 5, 6, 6, 6, 6, 6, 6, 8, 10, 14, 28, 38.	8.5
Controls without food (water only)	3, 4, 4, 4, 4, 4, 4, 4, 5, 5.	4.1

mals with greater food reserves to ascertain if they could adapt themselves to the high protein diet in time to benefit from it; one series was carried out on rats of moderate size (100–150 grams) and another on large rats (150–250 grams). The results are shown in Table 2. The medium sized rats gave a somewhat improved performance on the pure protein diet, their survival being about three-fourths as long as the fat- and carbohydrate-fed animals, in contrast to the small animals who lived only half as long on protein. The large animals actually survived longer on the protein diet, in fact nearly twice as long as the animals on fat or carbohydrate. It thus appears that if the rat is given time to adapt himself to an all-protein diet the superiority of that diet

becomes apparent. Large rats require ten days or more before they will take a diet of pure protein in quantity (Figure 2).

In a second group of experiments the attempt was made to adapt small rats (63–68 grams) to a high protein diet by gradually increasing the proportion of protein in their food from the time of weaning up to the onset of the single foodstuff diet.[3] By this means it was found possible to increase the survival time of small rats on casein alone from an average of 8.5 days to an average of 17.2 days. Individual rats in this group survived as long as those on carbohydrate or fat.

In Figure 1 is shown the caloric intake of a group of small rats on carbohydrate alone, a group on fat alone and a "protein adapted" rat

TABLE 2

Survival of Medium Sized and Large Rats on Single Foodstuffs

FOOD	MEDIUM-SIZED RATS (100–150 GRAMS)		LARGE RATS (150–250 GRAMS)	
	Survival time of individual rats	Average survival	Survival time of individual rats	Average survival
	days	*days*	*days*	*days*
Glucose	29, 33, 33, 42	34	32, 37, 37, 41, 41, 42, 43	39
Olive oil	32, 37, 39, 43	38	45, 59	52
Casein	7, 15, 19, 26, 30, 32, 33, 37, 43, 43, 45	30	51, 60, 80, 112, 123, 147	96

on protein alone that survived for an identical period of time. It will be noted that the caloric intake of the fat-fed and carbohydrate-fed groups is virtually identical. The protein-fed animal, in spite of his previous adaptation, showed a somewhat inferior food intake during the first week of the single foodstuff diet, but subsequently his caloric intake followed a course quite similar to that of the carbohydrate- and fat-fed animals.

What deductions can be drawn from these studies as to the merits of fat, carbohydrate and protein food in starvation? In the first place, it is apparent that adaptation is required if any foodstuff is to be handled in increased quantity. The time required for such adaptation is brief in the case of carbohydrate and fat. In the case of protein, not only is the process of adaptation a far slower one, but it is one that

[3] The final diet preceding the shift to casein only consisted of approximately 75 per cent of protein, two-thirds of which was casein.

cannot be accomplished by a young organism with limited tissue reserves,[4] for the reason that the utilization of additional protein requires the construction of new tissue.

An examination of the water intake of the animals on single foodstuffs (Figure 1) provides interesting information. On a diet of protein alone it will be noted that the intake of water is high, and furthermore, that the water intake shows a definite parallelism to the food intake.

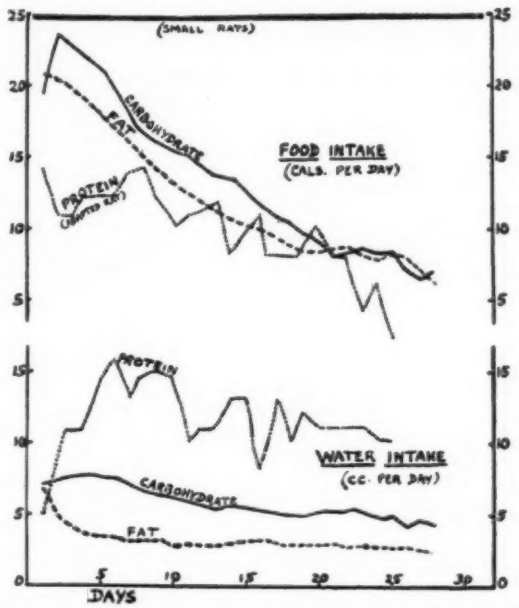

FIG. 1. FOOD AND WATER INTAKE ON PURE FOODSTUFFS

We have noted this phenomenon in all the rats fed upon protein alone—both large and small. The correlation between water intake and protein intake is very close when individual rats are considered (Figure 2). The animals fed on carbohydrate consume somewhat less water than those fed on protein, but a parallelism between the quantity of food

[4] The tissue reserves of the young animal are proportional to his body weight, but because of his relatively high metabolic requirements he is less well provided with a reserve to withstand starvation.

taken and the water intake is still apparent. In striking contrast is the behavior of animals on fat; their water intake was minimal and almost constant throughout the experiment, showing little relation to the quantity of food ingested.

In Table 3 are given data on the average food and water intake of a larger number of rats fed on single foodstuffs. The rats on carbohydrate only include groups of animals on glucose, levulose, invert sugar, maltose and sucrose; the rats on fat alone include groups of animals on the nine different edible fats which are discussed below. The data

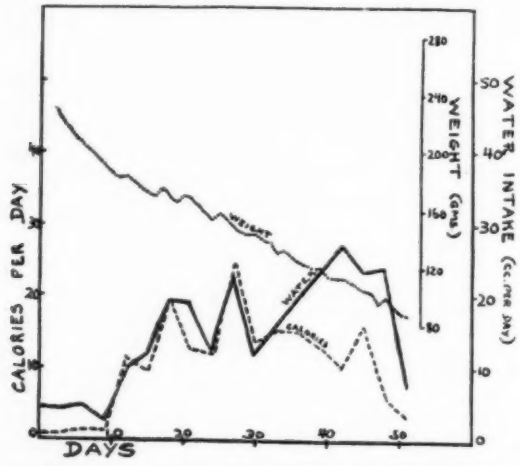

FIG. 2. WEIGHT, FOOD AND WATER INTAKE OF RAT ON CASEIN

in the Table support the conclusions reached above. The surplus water required by different foodstuffs (i.e. water taken in excess of that taken by fasting animals) is 0.85 cc. per calorie of ingested protein food, 0.29 cc. per calorie of ingested carbohydrate and only 0.08 cc. per calorie of ingested fat.

The need for water on a pure protein diet is easily explained by the increased quantity of urea to be excreted (6). The reason for the demand for water on the part of the carbohydrate-fed animals as contrasted with fat-fed animals is not quite so obvious, but is in keeping with numerous other observations that have been recorded. It is a

phenomenon familiar to pediatricians that an increase in the carbohydrate of the food causes a sudden gain in weight, due to the retention of water and electrolytes, until a new level is reached, after which the weight curve resumes its former slope. The reverse phenomenon is seen when fat is substituted for carbohydrate. The antiketogenic effect of carbohydrate, sparing the electrolytes and water used to excrete ketone bodies will not account for the water retention on carbohydrate since the phenomenon has been observed below the threshold of ketosis. Water stored with glycogen on a carbohydrate regime might cause a temporary demand for water on a newly instituted carbohydrate regimen, but would not explain a continuing demand for water.

TABLE 3

Water Intake of Small Rats on Single Foodstuffs

	[illegible] RATS	AV. FOOD INTAKE	AV. WATER INTAKE	WATER INTAKE PER CAL. OF FOOD	SURPLUS WATER INTAKE (BEYOND THAT INGESTED BY FASTING RATS)	SURPLUS WATER INTAKE PER CAL. OF FOOD	
		cals./day	*cc./day*	*cc.*	*cc./day*	*cc.*	
Protein only							
Casein (unadapted rats)	30	5.2	6.2	1.2	4.0	0.8	0.85
Casein (adapted rats)	9	10.0	11.8	1.2	9.3	1.0	
Carbohydrate only	102	13.1	5.9	0.45	3.7	0.28	
Fat only	221	12.0	3.1	0.26	0.92	0.08	
No food (water only)	6	0	2.2				

The phenomenon is not to be explained by differences in the water produced by the combustion of fat and carbohydrate, for although gram per gram fat yields nearly twice as much water as carbohydrate, calorie for calorie the water of combustion is almost identical and it may be recalled that the animals in our experiment had a comparable caloric intake. It is possible that differences in the physical activity of animals on a fat and a carbohydrate diet may have played an important part in determining the water requirements. Richter (4) observed that animals on a pure carbohydrate diet maintained a high degree of activity whereas animals on a fat diet were very inactive.

Regardless of the explanation, however, the fact remains that animals on a pure fat diet consumed, and apparently required decidedly

less water than on a carbohydrate diet and very much less than those on a protein diet.

The varying quantities of water demanded for the utilization of the different foodstuffs are of no concern when water can be freely supplied but under conditions of water scarcity this may become a matter of vital importance.

One further deduction may be drawn from these experiments on single foodstuffs. It may be recalled that when rats were shifted from a stock diet to one of carbohydrate alone the intake of the new food increased to a maximum within a few days, but shortly after this a decline in food intake set in which continued steadily until the death of the animal. The same phenomenon occurs, though less regularly, at the onset of a pure fat diet. The increase in food intake during the early days of the single food diet may represent the time required for the animal to adapt himself to the new diet so that he may utilize it in adequate quantities. The subsequent fa[illegible]e of appetite suggests the development of some deficiency which will no longer permit the animal to utilize the food in the quantities he has previously been able to handle. This conclusion seems inescapable if one grants that the appetite of the rat is conditioned by what he can utilize. It may well be that before the animal dies more than one deficiency develops, but it would appear that at least one deficiency has developed within a week of the onset of the experiment, a deficiency which limits the ability of the animal to utilize both carbohydrate and fat. Our attempts to throw light on the nature of this deficiency by supplementing the single food diets with various factors are described in the following papers of this series.

We shall now present experiments in which comparisons were made of individual carbohydrates, fats and proteins from the point of view of survival.

II. The Survival of Rats on Various Carbohydrates

In Table 4 are shown data on the survival of small rats offered different carbohydrates as their sole food. It will be noted that the survival of animals on dextrose, levulose, invert sugar, sucrose and maltose is strikingly constant, the average for these groups being 27.3, 32.3, 28.3, 29.3 and 29.0 days respectively. The food and water

intake of these groups of rats was comparable to that of the animals on glucose (Figure 3 and Table 5). It would appear that all of these sugars are equally valuable and equally well utilized under the conditions of these experiments. Our preliminary studies (3) which had

TABLE 4

Survival of Rats on Various Carbohydrates Alone

FOOD	SURVIVAL TIME OF INDIVIDUAL RATS	AVERAGE SURVIVAL
	days	*days*
Glucose	10, 11, 21, 23, 24, 24, 25, 25, 26, 26, 26, 27, 27, 27, 28, 28, 28, 28, 28, 28, 29, 29, 29, 29, 29, 29, 31, 31, 31, 31, 31, 32, 32, 36, 36.	27.3
Levulose	26, 26, 27, 28, 29, 29, 30, 30, 30, 30, 31, 34, 34, 35, 38, 38, 39, 47.	32.3
Maltose* (sterile)	24, 26, 27, 27, 29, 30, 30, 31, 32, 34.	29.0
Sucrose	17, 20, 22, 22, 22, 24, 24, 25, 25, 26, 27, 27, 28, 28, 28, 29, 30, 31, 32, 33, 33, 34, 34, 36, 36, 40, 40, 47.	29.3
Glucose and levulose (equal parts)	23, 25, 27, 28, 28, 28, 30, 31, 31, 32.	28.3
Lactose	4, 5, 5, 5, 5, 5, 5, 5, 5, 6, 6, 6, 6, 6, 6, 6, 6, 6, 7, 7, 7, 7, 8, 9, 10.	6.1
Galactose	3, 4, 4, 4, 4, 4, 4, 4, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 6, 6, 6, 6, 6.	4.8
Xylose	4, 5, 5, 5, 5, 5, 5, 6, 6, 6, 6, 6.	5.3
Sorbose	6, 6, 6.	6
Sorbitol	6, 6, 6.	6
Mannitol	5, 6, 6, 6.	5.8
Pentaerythritol	5, 5.	5
Controls (water only)	3, 4, 4, 4, 4, 4, 4, 4, 5, 5.	4.1

* In some preliminary trials with maltose longer survivals were encountered. It was subsequently found that the 50 per cent maltose solutions given to these animals supported the growth of moulds. The use of sterile maltose solutions obviated this difficulty.

indicated a superiority of dextrose over levulose and sucrose were apparently based on an insufficient number of animals.

The other sugars studied show a distinctly inferior performance. Animals receiving xylose, sorbose, sorbitol,[5] mannitol[5] and penta-

[5] These sugar alcohols were studied at the suggestion of Dr. John C. Krantz, Jr., who kindly supplied the materials.

erythritol[5] lived only a day or so longer than controls receiving nothing but water. These sugars were all ingested in minimal quantities, and since they are abnormal dietary constituents it is perhaps not surprising that animals exhibited little appetite for them. On the other hand, there is evidence that failure to ingest these sugars in quantity was actually due to a limited tolerance rather than to a natural deficiency of appetite. Examination of Table 5 indicates that even the small

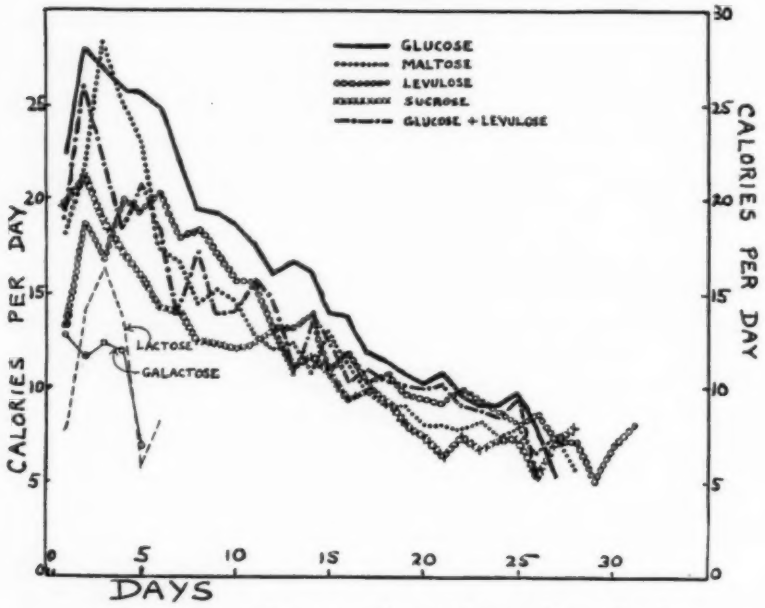

FIG. 3. CALORIC INTAKE ON VARIOUS SUGARS (SMALL RATS)

quantities of these sugars taken caused a marked increase in surplus water ingestion, far more than was caused by glucose and other well utilized sugars. This suggests an inability to utilize completely even the small amounts of sugar that were ingested and an added water requirement for the excretion of the unutilized surplus. Unfortunately, the urines of these animals were not examined for reducing substances.

The poor showing made by animals on lactose and galactose, which confirms our preliminary experiments (3) and those subsequently reported by Richter (4) was not to be anticipated and requires an explanation. As a group these animals ingested food in quantities comparable to those taken by animals on other well handled carbohydrates, although the performance of individual rats was far more erratic. In some instances very little sugar would be taken for a day or two followed by an enormous intake which at times exceeded 50 calories per day. Animals on galactose ingested enormous amounts of water. For

TABLE 5

Average Food and Water Intake of Small Rats on Carbohydrates Only

FOOD	NO. OF ANIMALS	FOOD INTAKE	WATER INTAKE	WATER INTAKE PER CAL. FOOD	SURPLUS WATER INTAKE (IN EXCESS OF THAT TAKEN BY FASTING RATS)	SURPLUS WATER INTAKE PER CAL. FOOD
		cals./day	*cc./day*	*cc.*	*cc./day*	*cc.*
Glucose	35	15.7	6.3	0.40	4.1	0.26
Levulose	18	12.4	7.2	0.58	5.0	0.40
Glucose and levulose	10	13.2	5.2	0.39	3.0	0.23
Maltose	19	11.2	5.5	0.49	3.3	0.29
Sucrose	20	11.0	4.8	0.44	2.6	0.24
Lactose	25	10.8	9.6	0.89	7.4	0.69
Galactose	25	11.7	24.4	2.1	22.2	1.9
Xylose	12	2.2	4.7	2.1	2.5	1.1
Sorbose	3	3.9	6.2	1.6	4.0	1.0
Mannitol	4	4.9	6.7	1.4	4.5	0.92
Pentaerythritol	2	2.5	7.2	2.9	5.0	2.0
No food (water only)	6	0	2.2			

the group as a whole, the average intake was 24.4 cc. per day; individual animals, however, consumed as much as 67 cc. in one day. At autopsy many of these animals were found to have enormously distended bladders occupying the major portion of the abdominal cavity. The urine from these animals was found to be strongly reducing. In order to identify the sugar in question a group of larger rats (150–175 grams) was placed upon galactose and urine was collected quantitatively in metabolism cages. It was found that the reducing substance appeared promptly as soon as the sugar was ingested in quantity and continued throughout the experiment. It was identified as galactose

by the mucic acid test.[6] Blood sugar determinations carried out on these animals showed levels between 200 and 550 mgms. per cent. A protocol of one of these large animals is shown in Figure 4.

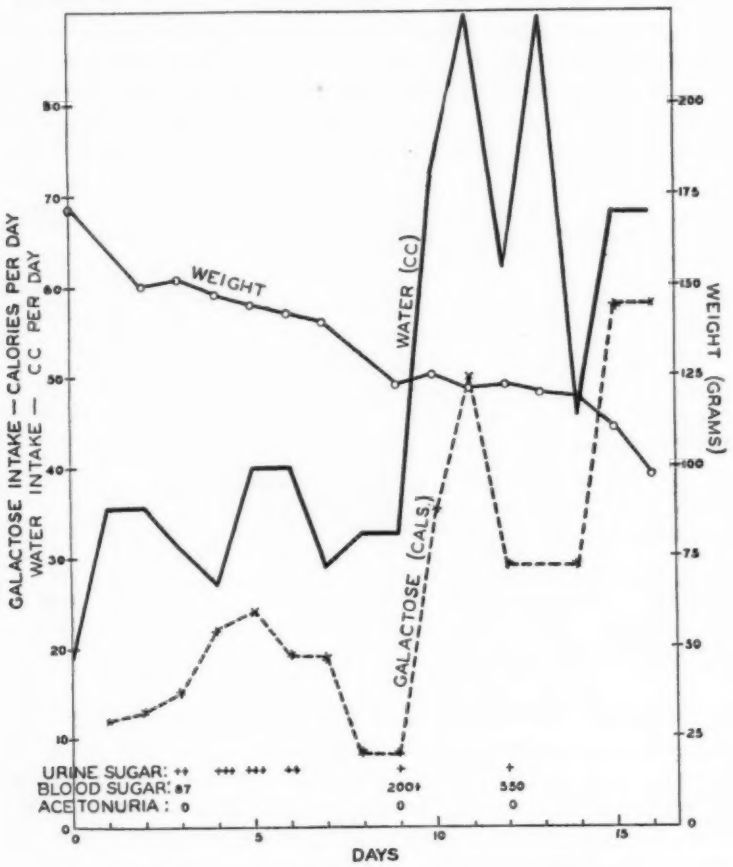

FIG. 4. EXPERIMENT WITH RAT ON GALACTOSE ONLY

It thus appears that the poor survival of animals on galactose must be attributed to their limited ability to utilize the quantities

[6] We are indebted to Dr. L. Kajdi for this determination.

of sugar they ingest. The excess sugar must be excreted in the urine, a process which requires water excretion. The condition may properly be described as a "galactose diabetes". In this case the appetite of the animal outruns his ability to utilize his food. It does not follow, however, that the rat's appetite for this sugar has been harmful in its net effect; possibly in spite of his defective mechanism he is burning more sugar than he would if he stayed within his tolerance.

It is well known that the tolerance of man and of many other animal species for galactose is less than that for glucose and levulose, but the differences observed have not been comparable to the striking difference observed in these rats maintained on single foodstuffs. At the time these experiments were carried out (1938) we were much puzzled by this phenomenon, but the observations subsequently published by Schantz and Krewson (7) may furnish an explanation for the greater difficulty in utilizing galactose when a pure carbohydrate diet is fed. These workers obtained evidence that the oxidation of galactose requires the presence of saturated fatty acids containing 12 or more carbon atoms. In view of the suggestion of Mason and Turner (8) that an excess of galactose might be responsible for liver damage in cases of idiopathic galactosuria, the liver was studied pathologically in several of our rats. No abnormalities of any kind were noted.

The behavior of rats on lactose was in general similar to that of animals on galactose with the exception that the appetite for water was less extreme. Many of the animals receiving lactose developed diarrhea, but the increased water consumption could not be explained by water lost from the bowel, for these animals also showed a marked diuresis. The urines of these animals were found to have strong reducing properties. The sugar was not identified, but it seems probable that it was galactose. Control tests for sugar were made on several animals receiving glucose or other well handled sugars, but in no instance was a reducing substance found.

The results obtained with lactose and galactose would certainly indicate a serious limitation in the value of these sugars in inanition states which demand their use in large quantities.

III. Survival Experiments on Different Fats

In Table 6 are shown our experiments on the survival of small rats fed different fats as their sole food. Data on the food intake of these

animals are given in Figure 5 and Table 7. The survival of animals on lard, olive oil and linseed oil is surprisingly constant, the average for these three groups being 28.5, 27.1 and 28.0 days, respectively, a survival time identical with that of animals on the well utilized sugars.

TABLE 6

Survival of Small Rats on Diets of Fat Alone

FOOD	SURVIVAL OF INDIVIDUAL ANIMALS	AVERAGE SURVIVAL
	days	*days*
Olive oil	14, 19, 21, 22, 22, 23, 23, 23, 23, 24, 24, 25, 25, 25, 26, 26, 26, 27, 27, 27, 28, 28, 28, 28, 30, 30, 31, 31, 31, 32, 32, 33, 34, 38, 41.	27.1
Olive oil + 10% halibut liver oil	16, 17, 18, 18, 19, 19, 24, 24, 24, 25, 28, 28, 28, 28, 28, 29, 29, 30, 30, 30, 30, 30, 30, 31, 35.	25.9
Olive oil + 10% wheat germ oil	18, 18, 19, 19, 21, 21, 22, 22, 23, 23, 23, 23, 23, 23, 24, 24, 24, 24, 24, 25, 25, 26, 26, 26, 26, 26, 26, 26, 26, 26, 26, 27, 27, 27, 27, 27, 28, 28, 29, 29, 29, 29, 29, 29, 29.	25.0
Lard	17, 19, 21, 22, 24, 24, 26, 26, 29, 29, 30, 31, 32, 33, 33, 33, 33, 34, 34, 34, 35, 36, 40.	28.5
Linseed oil	24, 24, 24, 25, 25, 26, 27, 29, 29, 29, 29, 30, 31, 31, 37.	28.0
Corn oil	19, 19, 20, 20, 20, 21, 22, 22, 22, 22, 22, 23, 24, 24, 25, 26, 26, 26, 26, 27, 27, 28, 28, 30, 30.	24.0
Cocoanut oil	15, 15, 19, 20, 21, 22, 23, 24, 26, 27, 27, 27, 28, 31, 34.	24.0
Peanut oil	14, 17, 18, 19, 20, 20, 22, 24, 25, 25, 27, 28, 29, 30, 31.	23.3
Cod liver oil	13, 17, 18, 20, 21, 21, 22, 22, 22, 23, 24, 24, 25, 25, 26, 27, 27, 27, 28, 29.	23.1
Sesame oil	12, 14, 16, 16, 17, 18, 21, 22, 23, 23, 24, 25, 25.	19.7
Rape seed oil	5, 6, 6, 7, 9, 10, 11, 12, 12, 13, 15, 16.	10.2
Controls (water only)	3, 4, 4, 4, 4, 4, 4, 4, 5, 5.	4.1

A slightly inferior survival time was noted on corn oil, cocoanut oil, peanut oil and cod liver oil (average—23 to 24 days). At the bottom of the list were sesame oil (19.7 days) and rape seed oil (10.2 days).

The reasons for the inferior performance of some of these fats are not altogether clear. There is some tendency to a decreased food

intake in the case of the fats that made the worst showing. A further factor is the fact that a number of the fats toward the lower end of the

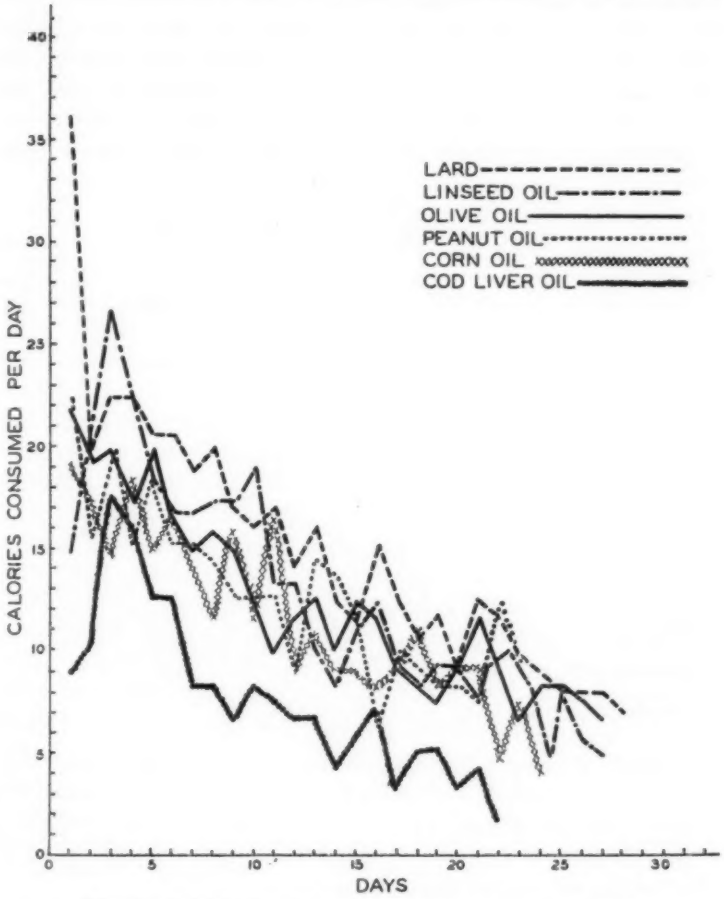

FIG. 5. CALORIC INTAKE OF SMALL RATS ON VARIOUS FATS

table contain appreciable quantities of fatty acids that are not readily assimilable. Peanut oil, for example, contains appreciable quantities of arachidic acid, a fatty acid with a longer carbon chain than is found in most edible fats. Although the oil is known to be well absorbed in

normal mixed diets it may be that on a high fat diet the quantity of this poorly absorbed constituent becomes significant. Even more difficult of absorption is the 24-carbon erucic acid found in rape seed oil. Cod liver oil likewise contains appreciable quantities of fatty acids not found in ordinary animal and vegetable fats, among them small quantities of odd carbon fats. A third possibility is that toxic factors in some of these fats may have contributed to the shorter survival time. Cocoanut oil contains considerable lauric acid, a fatty

TABLE 7

Average Food and Water Intake of Animals on Fat Alone

FOOD	NO. OF ANIMALS	AV. FOOD INTAKE	AV. WATER INTAKE	WATER INTAKE PER CAL. FOOD	SURPLUS WATER INTAKE (BEYOND THAT TAKEN BY FASTING RATS)	SURPLUS WATER INTAKE PER CAL. FOOD
		cals./day	*cc./day*	*cc.*	*cc./day*	*cc.*
Olive oil	35	12.1	3.2	0.26	1.0	0.08
Olive oil + 10% h. l. o.	25	12.4	3.6	0.29	1.4	0.11
Olive oil + 10% wheat germ oil	23	12.5	2.5	0.20	0.3	0.02
Lard	23	13.8	2.9	0.21	0.7	0.05
Linseed oil	15	12.8	3.8	0.30	1.6	0.12
Corn oil	25	12.0	3.3	0.28	1.2	0.10
Cocoanut oil	15	15.5*	3.2	0.21	1.0	0.07
Peanut oil	15	13.3	3.0	0.24	0.8	0.07
Cod liver oil	20	7.9	2.6	0.33	0.4	0.05
Sesame oil	13	11.1	3.0	0.27	0.8	0.07
Rape seed oil	12	9.0	3.2	0.35	1.0	0.11
No food (water only)	6	0	2.2			

* Figure probably too high; some spilled in cages.

acid which, if ingested in sufficient quantity, is known to cause toxic effects (9). In addition to toxic effects of particular fatty acids it is possible that nitrogenous materials present as impurities in some of these oils may have exerted some deleterious action. Further observations are needed to clear up these points.

IV. Comparative Observations on Single Protein Foods

It had been our intention to extend our preliminary observations of survival on pure protein diets (3) in order to determine accurately the relative value of different proteins in this situation. A number of such studies were, in fact, carried out before it was appreciated that we were

dealing with animals that had not had time to adapt themselves to a high protein intake. The survival time measured—a short one in all instances—actually did not furnish information about the nutritional value of the protein in question, but rather information about the adaptability of the animal to the particular protein fed. We believe that this criticism applies not only to our own published preliminary results (3) but also to the more extensive observations of Richter (4) whose animals were also not large enough to make a complete adaptation to a pure protein diet with one single exception. A further criticism of our preliminary observations is the fact that with the exception of casein, the proteins employed were of questionable purity.

Following the observation that large rats would survive on protein alone longer than on other single foods we carried out some comparative observations on casein and lactalbumin, using two brands of each, the purity of which was supposedly beyond question. In each instance the survival time with casein was longer than that with lactalbumin. This finding, together with the better showing made by casein in the incompletely adapted rats and in the animals studied by Richter (4) puzzled us, since lactalbumin has been regarded as having a higher biological value. The possibility that even lactalbumin especially purified for nutritional investigation might have been damaged in the course of preparation was difficult to escape. At a later date we had an opportunity to test a sample of lactalbumin which had been prepared by Dr. G. C. Supplee with particular care for some experiments carried out by Dr. A. A. Weech. A few rats given this preparation survived slightly longer than on casein. Since only a few animals were studied and the animals were large and hence likely to show considerable individual variation we do not feel warranted in making a statement about the comparative biological values of casein and lactalbumin in this type of experiment. This will require further studies which have not yet been undertaken, but we are relieved at not having to accept the conclusion pointed to by our earlier work that lactalbumin *per se* is an inferior protein.

SUMMARY

1. Reasons are given for believing that the nutritional requirements in inanition may be quite different from those that have been defined for normal growth and maintenance. The ability of single foodstuffs

to prolong survival has been utilized as an indicator of the requirement for particular foods in inanition.

2. Animals experience particular difficulty in adapting themselves to a higher protein intake than they have been receiving, apparently because the utilization of additional protein requires the construction of new tissue. Nevertheless, when conditions are such that an animal can adapt himself to a high protein intake he survives longer than on carbohydrate or fat, indicating that a deficiency of some protein factor comes into play at least in prolonged inanition.

3. Rats fed on fat or carbohydrate alone showed a gradual loss of appetite commencing usually toward the end of the first week and continuing progressively until death. This is attributable to loss of some essential dietary factor or factors concerned in the utilization of such food. The failure of appetite occurred at an identical time and progressed at an identical rate, regardless of whether the animals were fed fat or carbohydrate, which suggests that a factor required for the utilization of both foodstuffs gradually becomes depleted.

4. Striking differences were encountered in the water requirement on exclusively fat, carbohydrate and protein diets—proteins requiring the most water, carbohydrates next, and fats least of all. The reasons for these differences are discussed, and attention is called to the fact that *the "water-sparing" properties of carbohydrates, and more particularly of fats, may have practical importance in conditions of water scarcity.*

5. Marked differences in survival time were noted with different carbohydrates. The best results were obtained with glucose, levulose, maltose, sucrose and invert sugar—results which were practically identical. Pentoses and sugar alcohols are of negligible value under these conditions. Lactose and galactose made poor showings from the point of view of survival. The water requirement, particularly in the case of galactose, rose to enormous levels, owing to the fact that much of this sugar could not be utilized and required water for its excretion in the urine.

6. Differences were noted in the survival time on different fats; these were explained only in part by the chemical constitution of the fat.

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THE NUTRITIONAL REQUIREMENTS IN INANITION

II. The Effect of Mineral and Vitamin Supplements on the Survival of Animals on Single Foods[1]

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In the preceding paper (1) it was pointed out that the ability of single foods to prolong survival can be utilized to study the nutritional requirements in inanition. A number of such survival studies were reported. Among other things it was noted that animals fed on carbohydrate or fat alone exhibited a gradual failure of appetite, a phenomenon which was attributed to the development of some deficiency which impaired the animal's ability to utilize the food.

The present experiments were undertaken to throw light on the nature of this deficiency, the procedure employed being to supplement these single foods with minerals and vitamins. The experiments were carried out for the most part on small rats (60–70 grams) kept under the conditions described in the preceding paper. A few studies were made on larger rats, as is noted below. In some experiments a pure fat diet (olive oil) was used as a basis for supplementation and in others a pure carbohydrate diet (glucose) was employed. The food, the supplement and water were offered in separate containers, permitting the animals to select the quantity of each taken.

I. The Addition of Minerals to a Pure Carbohydrate Diet

Two experiments were carried out. In one series, the effect of supplementing a pure glucose diet with 3% sodium chloride solution was studied in small rats. In a second series, carried out on larger animals, the effect of a complete mineral supplement[2] was studied, the

[1] Aided by a grant from Mead Johnson and Company for the study of nutrition.

[2] The composition of this mixture, a slight modification of that devised by Cox and Imboden (2) was as follows: NaCl, 18.9 g.; $CaHPO_4$ (anhydrous) 25.0 g.; $MgSO_4$ (anhydrous) 6.86 g.; $KHCO_3$ 44.4 g.; KCl 2.88 g.; Fe_3 citrate (U.S.P.) 2.21 g.; $CuSO_4$ (anhydrous) 0.24 g.; $MnSO_4$ (anhydrous) 0.15 g.; KI 0.015 g.; NaF 0.03 g.

latter being offered in solid form. The survival data of these animals is shown in Tables 1 and 2.

It is apparent that neither the addition of sodium chloride nor that of a complete mineral mixture prolongs the life of the animals on a pure carbohydrate diet, indicating that under these conditions no mineral deficiency developed. It should be emphasized that this conclusion must be confined to the conditions of this particular ex-

TABLE 1

Effect of NaCl Supplement on the Survival of Rats fed Glucose only (65 gram rats)

DIET	SURVIVAL OF INDIVIDUAL ANIMALS	AVERAGE SURVIVAL
	days	*days*
Glucose	10, 11, 21, 23, 24, 24, 25, 25, 26, 26, 26, 27, 27, 27, 28, 28, 28, 28, 28, 28, 29, 29, 29, 29, 29, 29, 31, 31, 31, 31, 31, 32, 32, 36, 36.	27.3
Glucose and NaCl	13, 21, 21, 21, 21, 22, 22, 23, 23, 23, 24, 24, 25, 25, 25, 25, 25, 25, 26, 26, 26, 26, 26, 26, 26, 27, 27, 27, 27, 27, 27, 28, 28, 28, 29, 30, 30, 31, 46.	26.3

TABLE 2

Effect of Complete Mineral Supplement on Survival of Rats fed on Glucose Only (100–150 gram rats)

DIET	SURVIVAL OF INDIVIDUAL ANIMALS	AVERAGE SURVIVAL
	days	*days*
Glucose	29, 32, 33, 33, 42, 42.	35.2
Glucose and complete mineral mixture	27, 34, 34, 35, 35, 36.	33.5

periment. It is possible that conditions which permitted a more prolonged survival might have led to a mineral deficiency.

II. The Influence of B Vitamin Supplements on the Survival of Rats Fed only Carbohydrate

(a) *Thiamin.* Animals in this series were given access to a dilute solution of thiamin chloride (0.01 per cent). The addition of thiamin to the diet of glucose alone produced an extraordinary prolongation of

survival. Small rats on this regimen survived an average of 49.8 days, as contrasted with 27.3 days for animals on glucose alone (Table 3). It is thus apparent that a serious deficiency of thiamin develops in the course of a pure glucose diet and contributes to the early death of these animals. An examination of the composite curves for weight and food intake of these animals, as contrasted with the controls on glucose alone (Figure 1) shows no difference between the two during

TABLE 3

Influence of Various Vitamin B Supplements on Survival of Rats Given Glucose Only

DIET	SURVIVAL OF INDIVIDUAL RATS	AVERAGE SURVIVAL
	days	*days*
Glucose only	10, 11, 21, 23, 24, 24, 25, 25, 26, 26, 26, 27, 27, 27, 28, 28, 28, 28, 28, 29, 29, 29, 29, 29, 29, 31, 31, 31, 31, 31, 32, 32, 36, 36.	27.3
Glucose and thiamin	31, 32, 34, 34, 34, 34, 35, 38, 38, 38, 40, 40, 41, 42, 43, 43, 43, 45, 45, 45, 46, 47, 48, 50, 50, 52, 54, 54, 54, 55, 55, 56, 56, 56, 56, 57, 57, 57, 57, 57, 58, 58, 58, 58, 58, 58, 59, 60, 60, 62, 64, 66, 69.	49.8
Glucose and riboflavin	23, 24, 26, 28, 28, 30, 30, 30, 30.	27.7
Glucose and nicotinic acid	22, 29, 29, 32, 32, 33, 33, 33, 34, 35.	31.2
Glucose, thiamin, riboflavin, and nicotinic acid	34, 40, 43, 44, 45, 45, 47, 48, 49, 52, 52, 57, 57, 58, 62.	48.9
Glucose, thiamin, riboflavin, nicotinic acid, and pyridoxin	37, 42, 42, 46, 47, 48, 50, 51, 52, 53, 54, 54, 56.	48.6
Glucose, thiamin, riboflavin, nicotinic acid, filtrate factor concentrate	39, 43, 47, 50, 54, 56, 58, 61.	51.0
Glucose and vegex	35, 36, 37, 38, 42, 43, 44, 47, 48 49, 50, 50, 53, 59, 66.	46.5

the first two weeks of the experiment. Up to the thirteenth or fourteenth day, both sets of animals are losing weight and losing appetite at an equal rate, but from the fourteenth day onward, the loss of weight and appetite is less conspicuous in the rats receiving thiamin. These experiments permit the conclusion that thiamin deficiency appears only after a period of approximately two weeks on a pure carbohydrate diet. The early failure of appetite which appears on the third or fourth day must be due to some other factor.

Since our preliminary report of these studies was published (3) two other investigators have reported similar findings. Bollman (4), using rats partially depleted of B factors at the start of his experiments, showed that thiamin plus dextrose would permit a survival of

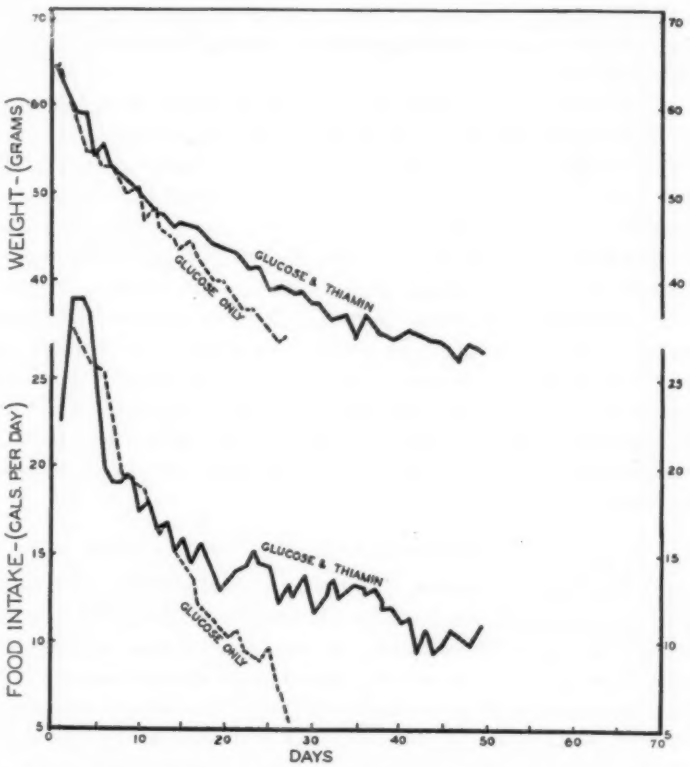

FIG. 1. INFLUENCE OF THIAMINE ON RATS RECEIVING ONLY GLUCOSE

two to four times that on dextrose alone. On the other hand, if his preliminary diet was rich in B factors no such prolongation was produced by thiamin. Richter and Rice (5) using somewhat larger rats (125–180 grams) were able to prolong their survival on dextrose from 37 to 74 days. Their thiamin fed animals diverged from the dextrose

controls some time between the tenth and twentieth day (intermediate points are not given in their report).

(b) *Riboflavin.* Experiments with rats given access to a dilute riboflavin solution (0.002 per cent) in addition to glucose are shown in Table 3. The survival time, food intake and weight curves of these animals did not differ from those of the glucose controls, in spite of the fact that these animals regularly took small amounts of the riboflavin solution.

(c) *Nicotinic Acid.* Rats offered a supplement of nicotinic acid showed a slightly longer survival than those on glucose alone (31.2 days instead of 27.3 days), a difference which is perhaps significant. The divergence of the curves of weight and food intake from those of the glucose controls occurred only at the end of the experiment. It is of interest to note that Bollman (4) also observed a slight prolongation of survival from a nicotinamide supplement in some of his experiments.

(d) *Multiple D Factors.* Several groups of animals were given access to various B factors in addition to thiamin, all of which were offered in separate containers. In none of these groups did the animals survive longer than with a supplement of thiamin alone, nor did their food intakes or weight curves differ appreciably from those of animals receiving only a thiamin supplement. Survival data for these groups are shown in Table 3.

III. The Addition of Vitamins to Pure Fat Diets

(a) *Fat Soluble Vitamins.* Two groups of experiments were carried out to see if the addition of fat soluble vitamins would prolong survival beyond that on olive oil alone. In one experiment a mixture of 90 per cent olive oil and 10 per cent halibut liver oil was used and in the other a mixture of 90 per cent olive oil and 10 per cent wheat germ oil was employed. The results shown in Table 4 indicate no appreciable difference in survival from similar animals given only olive oil. It would appear therefore that deficiencies of the fat soluble vitamins do not make their appearance under these conditions. This result is in harmony with the observations of Richter (6) on somewhat larger rats (100–150 grams) who found that animals on carbohydrate alone or fat alone developed no evidence of vitamin A deficiency in the vaginal smear up to the time of death.

(b) *Thiamin Deficiency.* In Table 4 are shown data on rats offered a thiamin supplement (0.01 per cent thiamin chloride solution) in addition to olive oil. It will be noted that the animals receiving thiamin lived slightly longer than the olive oil controls—32.0 days instead of 27.1 days. It is not necessary to conclude from this experiment that thiamin is concerned in the utilization of fat. Regardless of the controversy over the conversion of fatty acids into sugar, it is universally admitted that the glycerine fraction of fat (roughly 10 per cent) can be converted into sugar.

(c) *Riboflavin Deficiency.* Because of the suggestion made by Richter and Hawkes (7) and Mannering, Lipton and Elvehjem (8) that riboflavin might be concerned in the utilization of fat, the effect of adding such a supplement to a diet of olive oil was tried. Riboflavin was offered separately and was taken in small amounts regularly but produced no prolongation of survival.

TABLE 4

Effect of Vitamin Supplements on Survival of Rats on Fat Alone

DIET	SURVIVAL OF INDIVIDUAL ANIMALS	AVERAGE SURVIVAL
	days	*days*
Olive oil	14, 19, 21, 22, 22, 23, 23, 23, 23, 24, 24, 25, 25, 25, 26, 26, 26, 27, 27, 27, 28, 28, 28, 28, 30, 30, 31, 31, 31, 32, 32, 32, 32, 35, 36.	27.1
Olive oil and 10% halibut liver oil	17, 18, 19, 19, 20, 20, 25, 25, 25, 26, 29, 29, 29, 29, 29, 30, 31, 31, 31, 32, 32, 32, 32, 35, 36.	27.2
Olive oil and 10% wheat germ oil	18, 19, 21, 22, 23, 23, 24, 24, 25, 25, 26, 26, 26, 27, 27, 27, 27, 27, 29, 29, 29, 29.	25.1
Olive oil and thiamin	24, 27, 30, 33, 33, 34, 34, 34, 34, 37.	32.0

IV. Experiments in which both Carbohydrate and Fat were fed

It will be recalled that animals on fat alone and on carbohydrate alone showed a very striking parallelism in their food intake, which was characterized by a rising intake during the first day or two of the single food, followed by a gradual decline which continued until the death of the animal. In terms of calories, the food intake curves for the fat and carbohydrate fed animals could almost be superimposed,

which suggested the possibility that the factor causing the failure of appetite was identical in both cases. If such were the case, one would expect that rats offered carbohydrate and fat together would behave in a manner identical with that of rats on only one of these foodstuffs. On the other hand, if two distinct factors were concerned—one with fat utilization and another with carbohydrate utilization—it might be anticipated that some sparing action would be demonstrable and that rats offered both foods would give a better performance.

The data presented in Table 5 show that when thiamin was supplied the addition of fat to a diet of glucose alone caused no prolongation of

TABLE 5

Survival of Animals on Diets of Carbohydrate plus Fat, with and without Thiamin (60–70 gram rats)

DIET	SURVIVAL OF INDIVIDUAL ANIMALS	AVERAGE SURVIVAL
	days	*days*
Glucose only	See Table 1 of preceding paper (1)	27.3
Glucose and olive oil	30, 31, 31, 31, 34, 34, 35, 35, 35, 37, 37, 38, 39, 44, 49.	35.9
Glucose and 3% NaCl	See Table 1	26.3
Glucose, olive oil, and 3% NaCl	18, 20, 22, 22, 24, 24, 24, 24, 25, 26, 26, 26, 27, 27, 27, 27, 28, 28, 28, 28, 29, 29, 29, 29, 29, 29, 30, 30, 30, 32, 33, 33, 33, 34, 35, 37, 38, 38, 39, 39, 39, 40, 43, 44, 29.	30.0
Glucose and thiamin	See Table 3	49.8
Glucose, olive oil and thiamin	26, 33, 38, 48, 51, 53, 55, 57, 59, 60, 61, 66.	50.6

survival. The failure of appetite which commenced within a few days of the onset of the experiment was identical in both groups of animals receiving thiamin, and suggests that this is due to some factor involved in the utilization of both of these foodstuffs.

In the absence of thiamin, the addition of fat to a diet of glucose alone caused a slight, but definite, prolongation of survival, an effect which can be attributed to the thiamin sparing action of fat. The food intake curves of the animals receiving fat and carbohydrate, shown in Figure 2, are instructive. During the first two weeks of the experiment, the caloric intake of the animals was virtually identical with that of controls on glucose alone. During the third and more particularly, the

fourth week, however, the food consumption of the animals receiving both fat and carbohydrate tended to be better sustained, presumably because thiamin stores were less depleted than in the controls. During the early part of the experiment the animals regularly consumed more glucose than fat, but after the 25th day the rats on the 2-foodstuff diet showed a sharp falling off in their carbohydrate consumption, and a tendency to eat more fat. This presumably marks the onset of thiamin

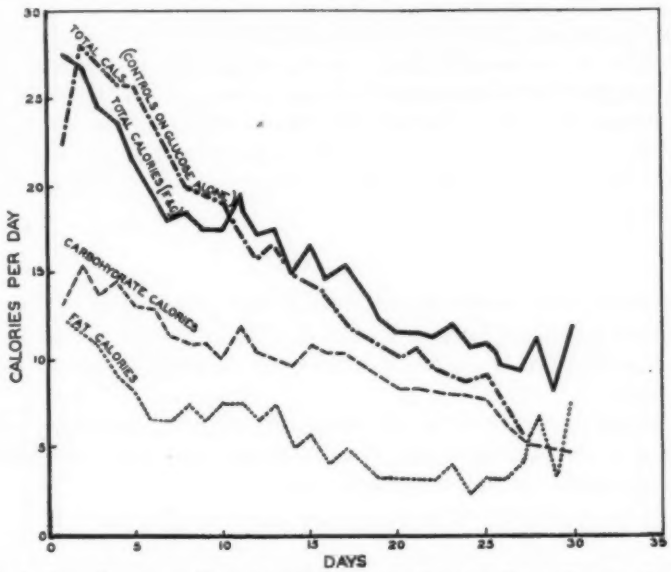

FIG. 2. FOOD INTAKE OF RATS GIVEN ACCESS TO GLUCOSE OLIVE OIL AND SALINE ONLY (65 GRAM RATS)

deficiency in them. The preference of the thiamin deficient rat for fat has been observed by Richter and ourselves in previous studies (10).

DISCUSSION

The foregoing experiments designed to throw light on the cause of appetite failure in rats fed on single foodstuffs—fat or carbohydrate—have shown that mineral deficiencies play no part in this phenomenon. Deficiencies of the fat soluble vitamins A, D, and E can likewise be

exonerated. Of the B vitamins, there is evidence that thiamin deficiency develops regularly in the third week; no clear cut evidence of deficiency of any other B factor was noted.

The most serious deficiency—one which causes failure of appetite as early as the third or fourth day—is apparently a deficiency of some factor that is needed for the utilization of fat and carbohydrate alike. The nature of such a factor is a matter for speculation at the present time. It may be some undiscovered accessory factor or it may be the protein component of some enzyme which cannot be synthesized in the presence of protein deficiency. It is noteworthy that when large rats are used (1) animals fed on protein alone maintain their appetite extraordinarily well in contrast to similar animals on fat and carbohydrate alone. Further experiments to elucidate the nature of this factor by means of protein supplements are reported in a subsequent paper (9).

SUMMARY

Survival experiments on rats indicate that a deficiency of thiamin develops relatively early in inanition. The stores of minerals, fat soluble vitamins, and other B factors are not subject to such early depletion.

Evidence is obtained of still earlier depletion of some factor concerned in the utilization both of carbohydrate and of fat, the nature of which is not clear at the present time.

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PROCEEDINGS OF THE MEETING OF THE JOHNS HOPKINS MEDICAL SOCIETY

Held in Hurd Hall, January 10, 1944

1. Observations on The Thiamine Requirements of Man. Drs. L. Emmett Holt, Jr., and Victor A. Najjar.

SUMMARY

The view that thiamine deficiency is widespread in the United States is based on dietary surveys which have revealed intakes of thiamin below the requirements established by the National Research Council. These requirements are open to question. They are based on experimental studies in which natural foods were used and it is known that natural foods vary widely in their thiamin content and that a considerable error is present in thiamin assays of food. Furthermore, the symptoms of deficiency observed in such studies were, in many instances, non specific.

The authors attempted to avoid these difficulties by the use of a synthetic diet in which vitamins were supplied exclusively as pure substances, in constant weighed amounts. The experimental diet which was fed to 9 adolescent and young adult males consisted of vitamin-free casein, crisco, dextrimaltose, a mineral mixture and a vitamin mixture. This diet, fed for 18 months without intermission, was identical for each of the 3 meals with the exception of the thiamin content which was gradually reduced from 1.0 mg. per day. The urinary excretion of thiamin virtually disappeared when the daily thiamin intake fell below 0.4 mg. per day, but no symptoms were observed even when it was reduced to 0.1 mg. per day. When the intake was reduced to zero, within 7 weeks four of the nine subjects developed unquestionable evidences of deficiency, which included frank neuritis, edema, tachycardia and changes in disposition. One or more of these symptoms was present in each of these four subjects, and loss of appetite was conspicuous in all four of them. One other subject presented doubtful manifestations and four others were symptom-free at this time. An examination of the stools showed that the four symptom-free subjects had an abundance of free thiamine in their stools; less was present in the subject with questionable symptoms, whereas the subjects with definite symptoms had almost no free thiamine in their stools. Evidence was obtained that the intestinal bacteria were producing the thiamine which was protecting the symptom-free subjects. Two subjects given sulfasuxidine showed disappearance of free thiamine from the stools within a week.

In spite of the synthesis of thiamine by intestinal bacteria, it appears that some thiamine is needed in the diet, for ultimately all but one of our subjects developed symptoms of deficiency. Probably some thiamine is necessary for the growth of the bacteria that produce thiamine. The composition of the diet appears to be a factor affecting the bacterial synthesis of thiamine.

It was concluded (1) that thiamine requirements are far lower than has been supposed; (2) that a protective mechanism against deficiency (biosynthesis) not previously recognized has been clearly demonstrated; (3) that the thiamine requirement must be evaluated for each particular diet, since the degree of biosynthesis varies with the diet.

DISCUSSION

Dr. Longcope asked whether alcohol was permitted the experimental subjects and whether the differences in the development of symptoms could be attributed to this factor.

Dr. Holt replied that the subjects were not allowed the use of alcohol and that there was no evidence that they had obtained any.

Dr. Park expressed the opinion that the present studies were important because the experimental conditions were so rigorous. He inquired about animal evidence for intestinal synthesis of thiamin and other vitamins.

Dr. Holt called attention to observations made by various investigators which had demonstrated the synthesis of various B vitamins in the cecum of the rat and in the rumen of ruminant animals.

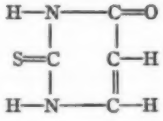
Dr. Rich stated that such studies should allay the fear that much of the population is suffering from dangerously low intakes of thiamine. He made the point that if mild subclinical vitamin deficiencies were as prevalent as some would have us believe, frank deficiency should be not too infrequent. Much has been made, for example, of the supposed prevalence of subclinical vitamin C deficiency in adults, and yet frank scurvy in the adult is very rarely seen.

2. Symposium on the Use of Thiouracil in Hyperthyroidism. DRS. ELLIOTT V. NEWMAN, WM. F. RIENHOFF, JR., AND ARNOLD R. RICH.

SUMMARY

Dr. Elliott V. Newman reviewed the discovery and development of the newer compounds which had been found to be effective against hyperthyroidism. These fell into 2 groups: (1) aniline derivatives of which p-, m- and o-amino benzoic acid were the most active, a group including the sulfonamides, and (2) the thiourea derivatives of which thiouracil was the most active. The latter compounds possess the chemical structure: —NH—CS—NH—, activity being lost if either NH group or S is replaced.

Thiouracil is



Following the favorable clinical reports of Astwood (3 cases) and of Williams (9 cases) in which complete remission of thyrotoxicity was obtained, this compound

was studied in six cases on the medical service of the Johns Hopkins Hospital in order to ascertain its usefulness in the preparation of patients for operation. The dosage employed was 0.6 grams daily. In each of these patients the BMR fell at about the same rate noted by Williams, reaching normal in 20 to 60 days. No uniform change in the size or consistency of the gland was noted. In only one instance was an untoward reaction observed; this patient after 10 days developed a febrile reaction which disappeared on stopping and reappeared on starting the drug again. She was then given Lugol's solution with further lowering of the BMR.

In comparing thiouracil with iodine from the clinical viewpoint, a number of differences may be noted. The *completeness of remission* differs, thiouracil causing a complete remission in every instance if continued long enough, whereas iodine does so in only 50% of the cases, another 30% to 40% showing partial remission and the remainder no effect at all. The *speed of remission* is much greater with iodine, which requires only 10 days for a complete response whereas thiouracil requires 20 to 60 days. Lastly, *recurrences* under thiouracil have not been observed; the metabolism is controlled as long as the drug is continued in contrast to iodine which controls the situation only temporarily. *Toxic effects* from iodine are mild and limited, but effects of a possibly serious nature have been observed with thiouracil including fever, edema of the ankles and in one case an exacerbation of exophthalmos.

Dr. Rienhoff commented upon the fact that the treatment of hyperthyroidism tended to run in cycles and perhaps we were reaching the end of the cycle of iodine enthusiasm. He was, however, not convinced that thiouracil was preferable to iodine. He showed sections of the gland of an iodine-fast patient whose response to thiouracil was at best minimal. In 2 cases he had observed a fall in blood cholesterol after thiouracil, a phenomenon he was at a loss to explain. As preoperative therapy iodine seems preferably to thiouracil; after the latter drug the gland remains more vascular and is a more difficult gland to operate on from the standpoint of hemostasis.

Dr. Rich pointed out that iodine and thiouracil act in different ways. Iodine causes an increase in colloid with flattening of the acinar cells. It perhaps acts by promoting the secretion into the acini rather than into the blood. When the acini have become distended, secretion into the blood may be resumed, explaining the temporary effect of this form of treatment which has in no way influenced the production of colloid. Thiouracil, on the other hand, leads in animals to a typical picture of hyperplasia. Animal experiments indicate that the formation of thyroxin is actually inhibited by the drug, and reduction of the basal metabolism should therefore be maintained by this therapy. The hyperplasia is attributed to an excessive secretion of thyrotropic hormone as a response to the lack of production of thyroxin. One cannot help wondering about the nature of this hyperplasia. If the cells are not secreting thyroxin, what are they doing that causes their hyperplasia? Can this be a case of hypertrophy and hyperplasia produced by a chemical stimulus (thyrotropic hormone) in the absence of increased work? Fur-

thermore, if the excretion of thyrotropic hormone is excessive, why is it that exophthalmos does not develop more regularly under the influence of thiouracil, since it is the thyrotropic hormone that is concerned with exophthalmos?

Dr. Longcope commented on the fact that we now have several methods available for the treatment of Grave's disease—operation, x-ray therapy, iodine and now thiouracil. The combination of iodine and operation has proved highly successful. There are, nevertheless, certain patients who cannot be operated upon, notably those with severe cardiac disease. In this group it seems possible that thiouracil will prove a great boon, but it would be surprising if it replaced iodine as preoperative therapy. Thiouracil is a compound which is important both from the practical and theoretical point of view and presents interesting facets for future study.

Dr. Blalock pointed out that none of the patients given thiouracil had been seriously ill. He stated that he would like to corroborate what Dr. Rienhoff had said about the shortcomings of thiouracil as a preoperative treatment. He doubted that it would replace iodine, but he thought it quite possible that some new drug would be discovered that would do so.

Dr. Wilkins inquired whether the storage of iodine in the thyroid after this uracil therapy had been studied by means of tagged iodine.

Dr. Rienhoff replied that studies carried out in Boston with radioactive iodine had shown that it was not stored in the gland during thiouracil therapy.

3. War Medicine on an Aircraft Carrier. CAPT. J. Q. OWSLEY, U.S.N.

Capt. Owsley gave a stirring account of his experiences, accompanied by motion pictures.

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of this Journal.)

Manual of Cardiology. By THOMAS J. DRY. 310 pp. Illus. $3.00. W. B. Saunders Company, Philadelphia and London, 1943.

This manual is intended as a simplified presentation of the field of cardiology. It is small and compact. It begins with a practical commonsense approach to the cardiac patient. A description of the normal heart is given and the symptoms and signs of the abnormal heart are discussed with a brief exposition of electrocardiographic changes. The various types of heart disease are described with the essential clinical features and management.

For this type of book, it is very readable. Some of the material is given in outline form, but most is in concise language. There are several x-ray and electrocardiographic illustrations of typical syndromes. There are a few minor blemishes such as a reversed electrocardiographic tracing and the misspelling of lanatoside throughout as in insecticide rather than as a glucoside. In regard to lanatoside and digitalis preparations, it is not made specifically clear how to proceed when switching from one to the other in the same patient. A few questionable forms of therapy are mentioned, such as fever therapy in chorea, and the efficacy of intraperitoneal mercurials over other routes of administration.

In the discussion of heart failure the definition of acute left sided failure and treatment of acute pulmonary edema are only touched upon in a short paragraph on cardiac asthma. Also perhaps too little emphasis is given to the accurate restriction of salt in congestive failure with edema, and too much to withholding water.

On the whole the manual can be recommended to the student or practitioner who wishes a brief readable survey of the field of cardiology written from a practical point of view.

E. V. N.

Burns, Shock, Wound Healing, and Vascular Injuries. Prepared under the Auspices of the Committee on Surgery of the Division of Medical Sciences of the National Research Council. 272 pages. Illus. $2.50. W. B. Saunders Co., Philadelphia and London, 1943. (Military Surgical Manuals, Vol. 5.)

This volume is the fifth in the series of Military Surgical Manuals. Although these books have been written primarily as a guide to the effective treatment of war injuries, much of the information contained within it may be of aid to the practitioner in the treatment of casualties unassociated with enemy action, since severe burns, extensive wounds, vascular injuries and various problems associated with wound healing are encountered even in time of peace. In many instances, however, the military situation imposes certain restricting factors which render impracticable

some procedures that would be considered ideal in civil life. The goal of furnishing the best possible treatment to all individuals is the same in the Army as in civil life, but the means to attain that goal may differ quite markedly.

The first one hundred pages deal with the *treatment of a severe burn;* from the moment of the initial injury when either first aid or definitive treatment is instituted, and subsequently, the reader is carried through the various steps or stages not only in the treatment of the burn surface itself, but in measures to prevent and control shock; the general care and welfare of the patient are outlined in detail. The proper application of the principles and measures outlined will reduce the need for much later specialized treatment.

The discussion of *shock* which comprises about 70 pages, is considered in 3 sections: (1) its nature and mechanism, (2) prevention and treatment, and (3) use of intravenous fluid therapy. Controversial details have been omitted, for the chapter has been prepared with the distinct realization that the prevention and treatment of shock must of necessity be carried out by medical personnel who are in immediate contact with the fighting forces.

The third chapter deals primarily with *"traumatic wounds"* which are always contaminated with bacteria and potentially infected. The technic of debridement of soft tissue wounds as well as compound fractures is outlined in didactic fashion together with the local and systemic use of the sulfonamides.

The last chapter is a splendid monograph on *vascular injuries and diseases* which is abundantly illustrated with line drawings simulating a surgical atlas. The site of election and technic of ligation of all the major vessels is beautifully illustrated and the reasons outlined in detail. The technics for sympathetic procaine blocks, sympathectomy, embolectomy, ligation and injection of varicose veins are well illustrated.

This fifth volume in the series prepared by recognized authorities under the auspices of the National Research Council and published by the W. S. Saunders Co. should serve as a ready source of information both for the civilian physician as well as those serving in the Armed Forces.

K. P.

Neurosurgery and Thoracic Surgery. Prepared and edited by the Subcommittees on Neurosurgery and Thoracic Surgery of the Committee on Surgery of the Division of Medical Sciences of the National Research Council. 310 pp. Illus. $2.50. W. B. Saunders Co., Philadelphia and London, 1943. (Military Surgical Manuals, Vol. 6.)

This compact little volume on neurosurgery and thoracic surgery is intended to be a guide for surgical treatment in the hands of surgeons who have not had the advantage of an intensive training in these specialties, and at the same time to standardize treatment in the most approved manner.

Such a manual can be of little practical value in substituting inexperienced surgeons for those properly trained.

The chapters on gunshot wounds of the head, of the spinal cord, and of thoracic surgery are excellent.

The chapter on intervertebral disks is already obsolete. This rapidly improved field—both in diagnosis and treatment—has been revolutionized since it was written by Dr. Love, who at that time was a master in this line. No longer are injections in the spine indicated for diagnosis—indeed they should not be tolerated. And in treatment the entire defective disk is now removed instead of merely removing the protruding portion. The improved treatment prevents recurrence which occurs with great frequency when the interior of the disk remains. Moreover, the high incidence of double and even treble disks makes their recognition at operation imperative.

The chapter on nerve injuries is much too diffuse, too extended, and in recommendations for treatment too equivocal.

A surgeon not familiar with this field could not see the forest for the trees. There is but one satisfactory treatment for nerve injuries, i.e., end to end suture with resection of the neuromata. To even consider nerve transplants, which probably are never successful, merely befuddles an inexperienced surgeon and, if attempted, probably loses for all time any probability of success. The advice to leave injured nerves six months to see what nature will do is very bad. Too much is at stake to leave anything to chance. Moreover, all nerve injuries with total loss of function should be inspected and, if severed, treated at base hospitals and as quickly as possible after the accident. Delay makes resection of neuromata necessary, and this creates a big hiatus between the ends of the nerve and these gaps create the great hazards in successful nerve surgery. The problem is really a simple one and unequivocal.

W. E. D.

Rehabilitation of the War Injured. By DOHERTY and RUNES. 684 pp. Illus. $10.00. Philosophical Library, New York, 1943.

This is a collection of reprinted papers dealing with Neurology and Psychiatry, Reconstructive and Plastic Surgery, Orthopedics, Physiotherapy, Occupational Therapy and Vocational Guidance and Legal Aspects of Rehabilitation.

While some of the subjects have a direct bearing on war injuries, many of the papers were published several years prior to the war and their applicability is frequently not obvious. The collection would be more valuable if it did not attempt to cover such a wide field of rehabilitation. As this volume is largely concerned with the surgical rehabilitation, the sections given over to Neurology and Psychiatry might well have been omitted in order to make room for expansion of the surgical aspects of the subject. Much will undoubtedly be learned in this war on rehabilitation and further publications on the subject will be awaited with interest.

E. M. H.

Medical Clinics on Bone Diseases. By I. SNAPPER. 225 pp. Illus. $10.75. Interscience Publishers, New York, 1943.

The book consists of discussions of the pathological physiology of five conditions affecting the skeleton—namely, hyperparathyroidism, deficiency of vitamin D, the lipoid granulomata, osteopsathyrosis and multiple myeloma. By far the largest proportion of the work is given to hyperparathyroidism. The author stresses the metabolic and medical aspects of these diseases which, by virtue of their skeletal symptomatology, are usually seen most by orthopedists rather than internists. Considerable emphasis is placed on vitamin D deficiency of which the author saw much in North China, but which is very rare in this country. The text is interspersed with many x-ray reproductions of skeletal lesions, most of which are of very high quality.

Several minor defects might be mentioned. In the opinion of the reviewer, case reports are sometimes incomplete; for example, patients are said to have edema without mention of blood pressure, cardiac status or renal function. The use of parathyroid extract is discussed in some places in terms of units, in other places as cubic centimeters. In the former, the older terminology of unitage is obviously meant, when 1 cc. was the equivalent of 20 units. The strength of a unit was reduced some years ago, and 1 cc. now equals 100 units. This might serve as a considerable source of confusion to those unfamiliar with this fact.

The importance of serum concentration in evaluating abnormalities of total serum calcium concentration is not given sufficient emphasis—in fact, is mentioned but once in the text. There is no discussion of one of the commonest bone diseases with which the clinician has to deal—namely, so-called "menopausal osteoporosis." The book might be more complete also if a brief discussion were included of conditions having increased bone density, such as marble bones and hypoparathyroidism.

However, the book is interestingly written in the author's delightful narrative style. On contraversial points it seems to the reviewer that quite fair representation is given to both sides. To internists and orthopedists the book should prove of interest and stimulation.

J. E. H.

A Synopsis of Clinical Syphilis. By JAMES KIRBY HOWLES. 671 pp. Illus. C. V. Mosby Company, St. Louis, 1943.

This textbook by Howles cannot be recommended to either medical students or practitioners, because of the presence of numerous errors, and major defects in the presentation and arrangement of material. Perhaps of greatest importance is the totally inadequate consideration of antisyphilitic therapy. For example, the recommended treatment for early syphilis, utilizing mercury, completely ignores accepted modern practice, while that for neurosyphilis is so replete with paradoxes and contradictions as to defy analysis. The recent literature is abstracted in a patchy, and often inaccurate, manner. Differential diagnosis is, in general, inade-

quately considered, in "laundry list" style. The sections on cardiovascular and on ocular syphilis are especially poor.

R. D. H.

The Ear, Nose, and Throat in the Services. By R. Scott Stevenson. 116 pp. Illus. $1.50. Oxford University Press, 1943.

Major Stevenson's book is not a reference text for the specialist in otolaryngology. Written as a manual for the use of the average medical officer, for this very reason it will also prove of value for the medical student or general practitioner. Therapy recommended is highly simplified because of the usual limitations both in drugs and instruments available to the average medical officer. There is little effort to describe the anatomy or physiology of the ear, nose, and throat, and there is no detailed differential diagnosis. The 112 pages of text are admirably concentrated on the essential simple facts of diagnosis and treatment of the common ear, nose, and throat ailments. There is no detailed section on ear, nose, and throat surgery.

The portion of the text devoted to chemotherapy seems inadequate from today's point of view. Almost nothing is said of the sulfa drugs used locally in the ear, nose, and throat, and nothing whatsoever about the use of the newer chemotherapeutic agents such as tyrothricin and penicillin. The use of sulfapyridine is overemphasized, and little mention is made of the value of sulfathiazole. There is no mention whatsoever of the extreme importance of continuing sulfonamide therapy for several days after the infection has been apparently cured. There is also no recommendation of the drugs in common use in this country as nasal vasoconstrictors, such as Neosynephrine and Privine Hydrochloride. Instead he advises such preparations as oily drops and Argyrol, with no discussion of the dangerous interference with ciliary activity by such drugs. Although there is an excellent section on external otitis, he does not mention the use of Cresatin (metacresylacetate) as a specific in the fungus infections.

Most of the book (60 pages) is devoted to the ear. Here in particular the reader will find excellent advice on avoiding superfluous and dangerous therapy. The section on hearing is a good one, but fails to emphasize the importance of masking. The importance of infected adenoid tissue in deafness or chronic ear infections is not stressed, although there is a very clear general statement on the importance of nasal infections in this relation. The value of inflation of the eustachian tube is overemphasized, and nothing is said of the potential dangers of this practice, particularly when used in aero-otitis. A longer and more detailed section on tracheotomy would have been desirable, and the mention of laryngotomy as a substitute for emergency tracheotomy should have been omitted. His recommendation of injecting cocaine into the trachea before incising is definitely bad advice.

On the whole, the average student or practitioner not intending to practice the more highly specialized techniques in otolaryngology will find this text simple, easily understood, and quite reliable, with the exceptions taken above.

D. F. P.